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GULF BREEZE
ENVIRONMENTAL RESEARCH LABORATORY

KEPONE IN THE MARINE ENVIRONMENT

PUBLICATIONS AND PREPUBLICATIONS

APPENDIX C

KEPONE MITIGATION FEASIBILITY PROJECT

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U.S. ENVIRONMENTAL PROTECTION AGENCY
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16. ABSTRACT

This report is Appendix C of the EPA report "Mitigation Feasibility for the Kepone Contaminated Hopewell/James River Areas". The work reported in this appendix was conducted by the U.S. EPA Gulf Breeze Environmental Research Laboratory and the Virginia Institute of Marine Science for the EPA Kepone Mitigation Feasibility Project. The twenty-two experiments conducted included Kepone chronic and acute toxicities for a variety of marine animals and plants, biological uptake by organisms and transfer of Kepone through food chains. Also covered are Kepone effects on microorganisms and the role of sediments in the storage, movement and transfer of Kepone in estuarine environments.

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EPA FOREWORD

This report has been prepared as one of the inputs to the Environmental Protection Agency's Kepone Mitigation Feasibility Project. The funded participants included: The Corps of Engineers, Norfolk District; the Department of Energy; Battelle Pacific Northwest Laboratories; EPA's Gulf Breeze Environmental Research Laboratory; and the Virginia Institute of Marine Science. The separate reports of the participants appear as Appendices to the Kepone Mitigation Feasibility Project Report.

While conclusions and recommendations are included in each participant's report, they are conditional within the scope of each project participant's tasks. Project conclusions and recommendations are the responsibility of EPA and are included in the Kepone Mitigation Feasibility Project Report.

The Executive Summary addresses the concept of Kepone "indices" development for water, for sediment, and for food consumed by organisms. At this time no formal criteria have been approved by EPA for Kepone. Thus, the "indices" discussed here should be considered only as "guides" for use in the Kepone Mitigation Feasibility Project.

U.S. Environmental Protection Agency
Office of Water and Hazardous Materials
Criteria and Standards Division
Washington, D.C. 20460

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EXECUTIVE SUMMARY

Prepared By

U.S. Environmental Protection Agency
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Gulf Breeze, Florida

May 9, 1978

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I. Conclusions

Laboratory studies indicate that Kepone does not degrade either biologically or chemically in simulated estuarine systems and this information suggests that degradation processes will not significantly alter the levels of Kepone now found in the water and sediments of the James River.

Clean-up indices for water quality were devised for Kepone in estuarine waters following the "Guidelines for Developing Water Quality Criteria for Aquatic Organisms for Consent Decree Chemicals." The final index for 24-hour average concentrations of Kepone in estuarine waters should never exceed 0.008 ug Kepone/1 of water.

Concentrations of Kepone in food organisms should be less than 0.015 mg Kepone/kg in tissue to minimize undesirable impacts on consumer species. Examination of partition coefficients between sediment and water, bioconcentra tion factors for benthic organisms, and food chain effects indicate that concentrations should be less than 0.015 mg Kepone/kg in sediment.

Conventional methods of analysis for Kepone in estuarine water, biota, and sediments have acceptable limits of detection of 0.020 ug Kepone/1 of wate 0.020 mg Kepone/kg in tissue and 0.020 mg Kepone/kg in sediment, respectively. The proposed criteria are below acceptable limits of detection by conventional methods of analysis and lead to the final conclusion that a hazard exists if Kepone can be detected in the James River Estuary by these methods of analysis

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II. Introduction

The Gulf Breeze Laboratory was initially involved with the Kepone problem in the James River Estuary late in 1975 with the initiation of studies to determine the toxicity of Kepone to estuarine animals, bioaccumulation of Kepone in estuarine animals, and studies of Kepone residues in animals living in the James River Estuary. These studies were gradually increased in scope during 1976 and some of the information on Kepone toxicity was presented in the federal criminal action brought against Allied Chemical Corporation and Life Science Products in October 1976.

During 1976 ongoing studies at ERL, Gulf Breeze, and research studies supported by ERL, Gulf Breeze, provided information on the ecosystem effects of Kepone and processes that were allowing crabs, oysters, and fish to concentrate Kepone in their tissues to a level making them unsafe for human consumption, i.e., reaching concentrations above the FDA designated action levels. This required a complete study of the effects of Kepone on representative species amenable for study under laboratory conditions, and to correlate these studies with all the information that could be gathered from monitoring and field experiments. The results of this information were used to develop saltwater indices for Kepone. The index for water was developed using the methods outlined in the "Guidelines for Developing Water Quality Criteria for Aquatic Organisms for Consent Decree Chemicals" prepared by the Ecology Laboratories of the Office of Research and Development. These guidelines are still evolving and the procedures used were current in April 24 1977. The criterion should include consideration of production, use, chemical, and physical properties, occurrence, and human health implications. However, our indices only considered protection of aquatic life and uses of aquatic life. Also, the unique situation in the James River Estuary where production and use have ceased and the major source of Kepone is recycled Kepone from the estuarine

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sediments requires a modified approach, the development of separate indices for aquatic food organisms and for sediment.

Significant concentrations of Kepone are present in various phases of the estuarine system of the James River--in solution, Huggett (1977) in the sediment, Nichols and Trotman (1977) and in the food chai , Bender et al. (1977), particularly in fishes. The interrelationships, or more specifically, the transport, uptake, and release of Kepone, are thus affected by both physio chemical mechanisms, Huggett (1977), Garnas et al. (1977), as well as bio-ecol gical phenomena, Bahner et al. (1977), Bahner and Oglesby (1977). The physica chemical mechanisms include the hydrodynamic transport through the estuarine system, adsorption to and desorption from the suspended and bed solids, and th settling and resuspension of these solids, Huggett (1977), Nichols and Trotman (1977). The latter incorporates the assimilation and excretion routes through the various components of the food chain, Bahner and Oglesby (1977), Schimmel et al. (in press). The potentially significant transport and kinetic processes transfer to the atmosphere, photochemical oxidation, and biological degradatio are not significant for Kepone, Garnas et al. (1978).

The basic information required as a major contribution to the mitigation study was the development of a quantitative framework to evaluate the dynamics of Kepone movement within the James River Estuary system. This could lead to an evaluation of the time required to reduce the Kepone concentrations to an acceptable level. The major role was taken by Battelle in providing this evaluation to the mitigation study and data from the experimental program and the field studies mentioned above have been used in the Battelle reports. The progress made by O'Connor and Farley (1977) in a parallel modeling effort is reported in this volume. The GBERL program has included a series of sponsored research projects at the Virginia Institute

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of Marine Science, principal investigator--Dr. Robert Huggett; Manhatten College, principal investigator--Dr. Donald O'Connor; Duke University, principal investigator--Dr. John Costlow; and Old Dominion University, principal investigator--Dr. Anthony Provenzano.

III. Water Quality Clean-up Index

Table I. Derivation of Saltwater Clean-up Index for Kepone

The reader is referred to the "Guidelines for Developing Water Quality Criteria for Aquatic Organisms for Consent Decree Chemicals" prepared by the Ecological Effects Laboratories of the Office of Research and Development in order to better understand the following summary and recommendation. The use of these "Guidelines" results in the following Tables and the calculations contained in them. These "Guidelines" were developed exclusively for the "65-21 Consent Decree Chemicals." Our use of the "Guidelines" for the establishment of a Kepone index is tentative, and should not be considered final or precise. Additionally, the Kepone index has been reviewed only at ERL, Gulf Breeze and has not received proper review by all ORD Ecological Research Laboratories and the Office of Water and Hazardous Materials as required for criterion generation.

The estuarine fish, spot *(Leiostomus xanthurus)* is particularly sensitive to Kepone; the Final Fish Acute Value calculated from this species is 6.6 ug Kepone/l of water. The Final Invertebrate Acute Value is 0.60 ug Kepone/l of water. Consequently the lower of the two, 0.60 ug Kepone/l of water, becomes the Final Acute Value.

Chronic studies have been conducted on sheepshead minnows and marine mysids. The Final Fish Chronic Value is <0.01 ug Kepone/l of water; the Final Invertebrate Chronic Value is 0.008 ug Kepone/l of water. Therefore, the Final Chronic Value is $\leq$0.008 ug Kepone/l of water.

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The marine alga, *Chlorococcum* sp., is the most sensitive plant species to Kepone; the Final Plant Value is <350 ug Kepone/l of water.

The Residue Limited Toxicant Concentration (RLTC) is based on (1) a study in which blue crab survival or molting was adversely affected after being fed a diet of oysters which contained 0.15 mg Kepone/kg in tissue, and (2) an average bioconcentration factor of 7688. This RLTC is <0.019 ug Kepone/l of water.

CLEAN-UP INDEX: The 24-hour average concentration should never exceed 0.008 ug of Kepone/liter of water.

It is important to emphasize that the data on the chronic effects of Kepone on fish, and the feeding studies on blue crabs provide "less than" values. Results of laboratory tests with crabs, shrimp, fish, and shellfish exposed only to Kepone in seawater underestimate the residues of Kepone measured in similar animals exposed to similar measured concentrations in the James River Estuary. Therefore, we consider that the index is conservative.

Table II. Fish Acute Values for Kepone

Organism	Bioassay Method *	Test Conc. **	Time (hrs.)	LC50 (ug/l)	Corrected LC50 (ug/l)	Reference
Sheepshead minnow, *Cyprinodon variegatus*	FT	M	96	69.5	69.5	Schimmel and Wilson, 1977
Longnose killifish, *Funuulus similis*	FT	U	48	84.	52.4	Butler, 1963
Spot, *Leiostomus xanthurus*	FT	M	96	6.6	6.6	Schimmel and Wilson, 1977
White mullet, *Mugil curema*	FT	U	48	55.	34.3	Butler, 1963

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*S = static; FT = flow-through

**M = measured; U = unmeasured

Geometric mean of corrected values = 30.1 µg/l $\frac{30.1}{3.7}$ = 8.14 µg/l

Lowest value from a flow-through test with measured concentration = 6.6 µg/l

Table III. Fish Chronic Values for Kepone

Organism	Test	Limits (ug/l)	Chronic Value (ug/l)	Reference
Sheepshead minnow, *Cyprinodon variegatus*	Embryo/ larval	<0.08	<0.08	Hansen, et al., 1977

Lowest chronic value = <0.08 µg/l $\frac{<0.08}{6.7}$ = <0.01 µg/l

Table IV. Invertebrate Acute Values for Kepone

Organism	Bioassay Method *	Test Conc. **	Time (hrs.)	LC50 (μg/l)	Corrected LC50 (μg/l)	Reference
Eastern oyster, *Crassostrea virginica*	FT	U	96	57.	43.9	Butler, 1963
Eastern oyster, *Crassostrea virginica*	FT	U	96	15.	11.6	Butler, 1963
Mysid shrimp, *Mysidopsis bahia*	FT	M	96	10.1	10.1	Nimmo, et al., 1977
Grass shrimp, *Palaemonetes pugio*	FT	M	96	120.	120.	Schimmel and Wilson, 1977
Brown shrimp, *Penaeus aztecus*	FT	U	48	85.	28.1	Butler, 1963

*S = static; FT = flow-through

**M = measured; U = unmeasured

Geometric mean of corrected value = 29.6 μg/l $\frac{29.6}{49}$ = 0.60 μg/l

Lowest value from flow-through test based on measured concentrations = 10.1 μg/l

Table V. Invertebrate Chronic Values for Kepone

Organism	Test	Limits (ug/l)	Chronic Value (ug/l)	Reference
Mysid shrimp, *Mysidopsis bahia*	LC	.026 to 0.072	0.043	Nimmo, 1978

Lowest chronic value = 0.043 µg/l $\frac{0.043}{5.1}$ = 0.008 µg/l

Table VI. Plant Effects for Kepone

Organism	Effect	Concentration (µg/l)	Reference
Alga, *Chlorococcum* sp.	Growth inhibition (EC50)	350	Walsh, et al., 1977
Alga, *Dunaliella tertiolecta*	Growth inhibition (EC50)	580	Walsh, et al., 1977
Alga, *Nitzschia* sp.	Growth inhibition (EC50)	600	Walsh, et al., 1977
Alga, *Thalassiosira pseudonena*	Growth inhibition (EC50)	600	Walsh, et al., 1977
Natural phytoplankton communities	94.7% decrease in productivity; 1,000 ^{14}C in a 4-hr exposure	1,000	Butler, 1963

Lowest Plant Value = <350 µg/l.

Table VII. Residues for Kepone

Organism	Bioconcentration Factor	TIME (days)	Reference
Eastern oyster, Crassostrea virginica	9,354	19	Bahner, et al., 1977
Eastern oyster, Crassostrea virginica	9,278	21	Bahner, et al., 1977
Marine mysid, Mysidopsis bahia	5,962	21	Bahner, et al., 1977
Grass shrimp, Palaemonetes pugio	5,127	28	Bahner, et al., 1977
Grass shrimp, Palaemonetes pugio	11,425	28	Bahner, et al., 1977
Sheepshead minnow, Cyprinodon variegatus	7,200	36	Hansen, et al., 1977

Maximum Permissable Tissue Concentration

Organism	Action Level or Effect	Concentration mg/kg	Reference
Man	Shellfish	0.30	
Man	Fish	0.30	
Blue crabs	Food	<0.15	Schimmel, et al., In Press

Average bioconcentration factor = 7,688

Lowest residue concentration = <0.15 $\frac{<0.15}{7{,}688}$ = <0.0000190 mg/kg or <0.019 μg/l

Table VIII. Other Data for Kepone

Organism	Test Duration	Effect	Result (ug/l)	Reference
Alga, Chlorococcum sp.	24 hrs	Bioconcentration factor = 800X		Walsh, et al., 1977
Alga, Dunaliella tertiolecta	24 hrs	Bioconcentration factor = 230X		Walsh, et al., 1977
Alga, Nitzschia sp.	24 hrs	Bioconcentration factor = 410X		Walsh, et al., 1977
Alga, Thalassiosira Pseudonena	24 hrs	Bioconcentration factor = 520X		Walsh, et al., 1977
Eastern oyster (larvae), Crassostrea virginica	48 hrs	EC50	66.	Bionomics Report, 1976
Blue crab, Callinectes sapidus	4 days	Bioconcentration factor = 8.1		Schimmel and Wilson, 1977
Grass shrimp, Palaemonetes pugio	4 days	Bioconcentration factor = 698		Schimmel and Wilson, 1977
Grass shrimp (larvae), Palaemonetes pugio	≤21.7	No effect on survival, size or duration of larval stages for larval with residues of <0.63 mg/kg Kepone		Provenzano, et al., 1977
Sheepshead minnow, Cyprinodon variegatus	4 days	Bioconcentration factor = 1548		Schimmel and Wilson, 1977
Spot, Leiostomus xanthurus	4 days	Bioconcentration factor = 1221		Schimmel and Wilson, 1977
Blue crab, Callinectes sapidus	96 hrs	20% mortality	1,000.	Butler, 1963
Blue crab, Callinectes sapidus	96 hrs	0% mortality	210.	Schimmel and Wilson, 1977
Blue crab, Callinectes sapidus	56 days	Survival or molting was reduced in crabs fed oysters containing 0.15 mg/kg Kepone		Schimmel, et al., In Press

IV. Food Clean-up Index

Acute exposure of blue crabs to Kepone in sea water in the laboratory indicated relatively low toxicity and bioconcentration, Schimmel et al. (1977). In contrast, monitoring data indicated that blue crabs from the James River Estuary accumulated significant concentrations of Kepone, Bender et al. (1977). Schimmel et al. (in press) found that the major route of Kepone entry is through contaminated food and not water. Therefore, estimates of an index for contaminated food were developed as shown in Tables I and VII of the water index. Guidelines have not been developed by OR&D laboratories for these types of data to estimate safe concentrations for untested species and to provide adequate protection when data do not support a no-effect concentration. We support a Kepone food index of 0.015 mg Kepone/kg of tissue obtained in the following manner.

Toxic Effects of Kepone in Food

Effects of Kepone on growth and survival of blue crabs fed oysters contaminated with Kepone, Schimmel et al. (in press), are the only laboratory data demonstrating adverse effects of this pesticide in food on an aquatic organism. Concentrations of 0.15 mg Kepone/kg of oyster meat fed to blue crabs diminished survival or molting. However, because the data do not provide us with a no-effect concentration, we applied a safety factor of 0.1 to this concentration to provide, in our judgement, an index (0.015 mg Kepone/kg of tissue) that should be protective of consumer species.

Food Clean-up Index Relative to Kepone in James River Food Organisms

A criterion of 0.015 mg Kepone/kg in food organisms is far less than monitoring data revealed in animals from the James River Estuary. An analysis of the data of Bender et al. (1977) indicated that the average concentration of Kepone in fishes and invertebrates from the James River which could be eaten ranged from

0.090 to 2.0 mg Kepone/kg of tissue. Our data on effects of Kepone in oysters fed to blue crabs support the hypothesis that undesirable impacts on survival and molting of blue crabs are occurring in the James River.

V. Sediment Clean-up Index

The monitoring studies by Huggett (1977), the State of Virginia (1977), and Battelle (1977) clearly demonstrate that most of the discharged Kepone is now resident in the sediments of the James River Estuary. The main sink is in the bed sediment of the estuary, in the zone of the turbidity maximum, where suspended sediments are trapped and deposited. The hypothesis used to explain this distribution, and the processes used in modeling Kepone transport (and long-term persistence and bioavailability in the estuary), is that Kepone is bound to sediment and sediment transport processes control the distribution of Kepone, Huggett (1977), O'Connor (1977).

The concentrations of Kepone are orders of magnitude greater in the bed sediments than dissolved in estuary water. Garnas et al. (1978), Huggett (1977), and Battelle (1977) have shown that partition equilibria for Kepone between sediment and water are directly affected by the sediment quality. Therefore, mitigation must address Kepone in sediments.

It is important to use all the available information to estimate the sediment concentration that must be achieved to eliminate the hazard to aquatic life and its consumers. Additionally, it is essential to consider: 1) the direct toxic effects and the bioavailability of Kepone contained within the bed sediments, Bahner (1977), Rubinstein (1977), and adsorbed to suspended sediment, Haven and Morales-Alamo (1977); 2) bioconcentration in the food chain directly from sediment; and 3) the dissolved Kepone in the estuary water which is controlled by the sediment/water equilibrium, Garnas et al. (1978).

Indices for acceptable concentrations of Kepone in sediments can be derived by examining how Kepone partitions among water, sediments, and

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benthic biota. Experiments have shown that benthic organisms (lugworms, Arenicola cristata, and fiddler crabs, Uca pugilator) that injested James River sediments with 0.250 mg Kepone/kg of sediment attained whole-body residues of 0.250 to 0.300 mg Kepone/kg of tissue within 21 days, Bahner et al. (in preparation). Lugworms did not survive exposure to these sediments after 21 days. Concentrations as low as 2.8 ug Kepone/l seawater caused a reduction in the normal substrate reworking activity of the lugworm and 29.5 ug Kepone/l seawater was acutely toxic within 144 hours to lugworms burrowing in sediments, Rubinstein (1978). Kepone did not depurate from lugworms and fiddler crabs over a period of a few weeks in clean water, Bahner et al. (in preparation).

Clean-up Indices Formulation for Kepone in Sediments

Three methods for determining indicies for Kepone-laden sediments are feasible:

1. a safety factor can be applied to sediment concentrations shown to be toxic to animals;

2. food quality index for estuarine food chain based on accumulation of Kepone by animals from sediments to concentrations that exceed the food quality index;

3. index based on desorption of Kepone from James River sediment into water at concentrations that exceed the water quality index;

Sediment Clean-up Index Based on Sediment Toxicity and Safety Factors

Insufficient data are available to determine if reducing Kepone concentrations in sediment by any factor will be protective to benthic organisms.

Sediment Clean-up Index Obtained from the Food Quality Index

Benthic organisms attained Kepone concentrations similar to the amount in sediments. The food quality index is 0.015 mg Kepone/kg of tissue. Therefore, Kepone concentrations in sediment should not exceed 0.015 mg Kepone/kg of sediment to insure that Kepone concentrations are less than the food quality index.

Sediment Clean-up Index Obtained from the Water Quality Index and Sediment/Water Partition Coeffients

Establishment of an acceptable concentration of Kepone in sediments may be based upon the premise that an equilibrium exists for Kepone between the sediment and water [Kp = (ug/kg sediment)/(ug/l water)]. An examination of laboratory Kp-values indicates numbers ranging from 2.5 to 1700, Garnas et al. (1978), Huggett (1977), Dawson (1977). If pure reference clays and sand are ignored (Kp = 2.5-50), the range is between 100 to 1700 and is related to the quality and quantity of organic material in the sediment. Using these values to derive acceptable sediment concentrations, a Kepone water index not greater than 0.008 ug Kepone/l of water at equilibrium is related to a range from 0.0008 to 0.014 mg Kepone/kg in sediment. The average concentration of Kepone in James River sediments from December 1976 through July 1977 was 0.150 mg Kepone/kg of sediment, Huggett (1977), with the limit of Kepone analysis in sediments at 0.020 mg Kepone/kg of sediment.

Derivation of a Final Kepone Sediment Clean-up Index

If a Kepone partition between water and James River sediment of Kp=1000 is utilized, concentrations of 0.008 mg Kepone/kg of sediment would result in equilibrium concentrations equal to the Water Quality Index of 0.008 ug Kepone/l of water. Because the food quality index is 0.015 mg Kepone/kg of tissue, the concentration of Kepone in sediment must not exceed 0.015 mg Kepone/kg of sediment. Since the lower limit of analytical detection of Kepone in sediments is usually 0.020 mg Kepone/kg of sediment, both derived concentrations are below analytical detection. Therefore if Kepone is present in measurable quantities, it is hazardous to aquatic life.

VI. Degradation of Kepone

Test Results

Studies by Garnas et al. (1978) have employed static and flowing water-sediment systems to assess both biological and non-biological degradation of Kepone. Sediments with and without Kepone contamination were taken from the James River and used in these systems. The fate of Kepone was monitored using radiolabelled (^{14}C) material and total budget chemical analysis. Using a variety of experimental conditions (oxygen concentration, nutrient additions, Kepone levels, sediment sources, sunlight, temperature, and salinity), these studies indicate that Kepone does not degrade (i.e., complete recovery of Kepone after extended incubation periods) either biologically or chemically in laboratory systems. These data suggest that degradation processes will not significantly alter the levels of Kepone now found in the water and sediment of the James River, and place further emphasis on the indices derived for water, food, and sediment.

Acute toxicity of KEPONE to embryos of the eastern oyster (*Crassostrea virginica*)

Toxicity Test Report
Submitted to
U.S. Environmental Protection Agency
Gulf Breeze Laboratory
Sabine Island
Gulf Breeze, Florida

Bionomics - EG&G, Inc.
Marine Research Laboratory
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March 1976

A marine toxicity test was conducted to determine the acute effect of KEPONE on embryos of the eastern oyster (Crassostrea virginica). The criterion for effect was reduction in the number of normal embryos in test concentrations (those which developed to the fully-shelled, straight-hinged veliger stage within 48 hours) as compared to the number of normal control embryos. Results of the test are expressed as a 48-hour EC50 (the concentration of KEPONE estimated to be effective in preventing normal development of 50% of the embryos).

MATERIALS AND METHODS

Test material

A 2.5 gram sample of a white powder was received in a small glass vial labeled "KEPONE, 88% pure, 2.5 g." Accompanying instruction from U.S. Environmental Protection Agency Research Biologist Steven C. Schimmel directed us to assume 100% purity for testing purposes. Test concentrations are reported here as micrograms (μg) of KEPONE per liter (ℓ) of sea water or parts per billion (ppb).

Test animals

Oyster embryos were obtained by induced spawning of oysters which had been conditioned in the laboratory for 6-8 weeks in flowing, unfiltered natural sea water at 25±2 degrees Celsius (°C).

Test conditions

Methods for the acute (48-hour) oyster embryo test were based on those of Woelke (1972) and U.S. Environmental Protection Agency (1975). Individual, sexually mature female oysters held in glass chambers containing 1 ℓ of filtered (5 micrometers, μm) sea water were induced to spawn by increasing the water temperature in the chamber from 25°C to 33°C over a 30-minute interval in the presence of viable sperm excised from the gonad of a sexually mature male oyster. Fertilization occurred upon release of the eggs into the spawning chambers. Microscopic examination confirmed fertilization success to be ≥95%. Density of the embryos was determined by averaging three 1-mℓ Sedgewick-Rafter counts of 1:99 dilutions (1 mℓ embryo suspension to 99 mℓ sea water) from the spawning chamber.

Concentrations for the definitive 48-hour test were based on a preliminary assay and were 56, 65, 75, 87, and 100 ppb. All test concentrations and the control were triplicated. Test containers were 1-ℓ glass jars, each containing 900 mℓ of filtered (5 μm), natural sea water. Test concentrations were prepared by pipetting appropriate amounts of KEPONE, dissolved in reagent grade acetone, into each test container. Control jars received 900 mℓ of sea water with no KEPONE. Salinity was 21 parts per thousand (^{o}/oo) and initial pH was 8±0.5 for all test concentrations and the controls.

For the definitive test, each test container was inoculated with an estimated 26,000±1,000 embryos and then maintained at 20±1°C in a controlled environmental chamber. After 48 hours of exposure, the embryos from each test container were collected by seive (37 μm), rinsed into a 100-mℓ graduated cylinder from which a 10-mℓ aliquot was transferred to a small glass bottle and preserved with 0.3 mℓ of neutralized formalin. The number of normally developed 48-hour embryo present in 1 mℓ of each preserved sample was determined by Sedwich-Rafter counts. The percentage reduction of normal 48-hour embryos was determined as follows:

$$\text{Percentage reduction} = \frac{\begin{array}{l}\text{Number of normal 48-hour control embryos}\\ \text{minus the number of normal 48-hour embryos}\\ \text{in each test concentration}\end{array}}{\text{Number of normal 48-hour control embryos}} \times 100$$

The number of normal embryos was based on the average of two counts of 1 mℓ of preserved sample from triplicate test concentrations and control.

Statistical Analysis

Based on results of the test, a 48-hour EC50 for eastern oyster embryos was calculated. The test concentrations were converted to logarithms and corresponding percentage reduction of normally developed 48-hour embryos to probits. The 48-hour EC50 was then calculated by linear regression.

RESULTS AND DISCUSSION

A preliminary test with oyster embryos showed the concentration of KEPONE effective in preventing normal development to be >56<100 ppb (Table 1). It also showed the volume of acetone used as a solvent/carrier did not affect normal development of embryos (Table 2). Further, it is interesting to note the increased number of normal embryos in the lower test concentrations (10, 32, and 56 ppb) of the preliminary test. We have observed this effect in tests with several different compounds and do not know whether it is caused by the presence of the toxicant, a synergistic effect between toxicant and carrier, or is random error.

The definitive test confirmed the results of the preliminary test. After 48 hours exposure, percentage reduction of normally developed exposed embryos as compared to the control was from 0% in the 56 ppb test concentration to 100% in 87 and 100 ppb (Table 3). The calculated 48-hour EC50 for eastern oyster embryos exposed to KEPONE in static, unaerated sea water was 66 ppb with 95% confidence limits of 60-74 ppb.

The average number of normal embryos counted per 1 mℓ of a 1:99 dilution of the embryo population collected from each test concentration ranged from 0 in 87 and 100 ppb to 152 in 56 ppb (Table 4). The 48-hour embryos from 56 ppb test concentrations appeared to be smaller and not as well formed as the control; however, they were fully shelled and straight-hinged. In 65 ppb, although only an average of 44 normal embryos were counted, there were numerous abnormal embryos present. The severity of abnormal development increased in the higher test concentrations of 75, 87, and 100 ppb.

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TABLE 1. The acute toxicity of KEPONE to embryos of the eastern oyster (Crassostrea virginica) exposed for 48 hours in static, unaerated sea water. Salinity was 21 ‰ and temperature, 20±1°C. The criterion for effect was the reduction in the number of normal embryos in test concentrations as compared to the number of normal control embryos.

Nominal concentration (µg/ℓ;ppb)	Percentage reduction of normal 48-hour embryos[a]
Sea water control	0
Acetone control	0
10	0
32	0
56	0
100	100
180	100

$$^{a}\text{Percentage reduction} = \frac{\text{Number of normal 48-hour control embryos minus the number of normal 48-hour embryos in each test concentration}}{\text{Number of normal 48-hour control embryos}} \times 100$$

TABLE 2. Number of normal eastern oyster (Crassostrea virginica) embryos per milliliter counted following 48 hours of exposure to KEPONE in static, unaerated sea water. If all embryos in the initial inoculum had developed normally, the expected count would have been 314±16 embryos per milliliter. Salinity was 21 ‰ and temperature, 20±1°C.

Nominal concentration (µg/ℓ;ppb)	Number of normal embryos		
	Rep A	Rep B	Avg.
Sea water control A	198	179	188
Sea water control B	179	175	177
Acetone control	160	212	186
10	206	253	229
32	285	280	283
56	264	236	250
100	0	0	0
180	0	0	0

TABLE 3. The acute toxicity of KEPONE to embryos of the eastern oyster (*Crassostrea virginica*) exposed for 48 hours in static, unaerated sea water. Salinity was 21 °/oo and temperature, 20±1°C. The criterion for effect was the reduction in the number of normal embryos in test concentrations as compared to the number of normal control embryos.

Nominal concentration (μg/ℓ;ppb)	Percentage reduction of normal 48-hour embryos[a]
Sea water control	0
56	0
65	68
75	97
87	100
100	100

[a]Percentage reduction = $\frac{\text{Number of normal 48-hour control embryos minus the number of normal 48-hour embryos in each test concentration}}{\text{Number of normal 48-hour control embryos}} \times 100$

TABLE 4. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 hours of exposure to KEPONE in static, unaerated sea water. If all embryos in the initial inoculum had developed normally, the expected count would have been 247±12 embryos per milliliter. Salinity was 21 ^{o}/oo and temperature, 20±1°C.

Nominal concentration (μg/ℓ;ppb)	Number of normal embryos			
	Rep A	Rep B	Rep C	Avg
Sea water control	128	138	145	137
56	151	147	158	152
65	42	43	46	44
75	2	8	2	4
87	0	0	0	0
100	0	0	0	0

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Table III. Fish Chronic Values for Kepone

Organism	Test	Limits (ug/l)	Chronic Value (ug/l)	Reference
Sheepshead minnow, *Cyprinodon variegatus*	Embryo/ larval	<0.08	<0.08	Hansen, et al., 1977

Lowest chronic value = <0.08 µg/l $\frac{<0.08}{6.7}$ = <0.01 µg/l

34<

Kepone: Hazard to Aquatic Organisms

Rudolph J. Jaeger (Letters, 9 July, p. 94) reports the chronology of mammalian toxicity tests with Kepone (chlordecone) and the exposures of workers at the Life Science Products Company of Hopewell, Virginia. Initial concern has properly been focused on the results of toxicity and carcinogenicity tests on rats, rabbits, dogs, and mice and on the disease that Kepone produced in exposed workers. We would like to document our concern about the hazard of Kepone to aquatic organisms in the James River and the Chesapeake Bay.

On-site tests of organisms taken from the James River showed significantly high Kepone concentrations. These tests, conducted at the Virginia Institute of Marine Science and funded by the Environmental Protection Agency (EPA), revealed that concentrations in edible tissues of most fresh and estuarine fin- and shellfish commonly ranged from 0.1 to more than 1 microgram per gram. These concentrations exceeded allowable health limits for commercial and sport fisheries and forced closure of the river to some commercial and sport fishing. This year Kepone concentrations have increased in anadromous fishes as they spent more time in the river.

Further, after laboratory exposures at the EPA laboratory in Gulf Breeze, Florida, we found that Kepone, like other chlorinated insecticides, is highly bioaccumulative and persists in estuarine organisms. Oysters, grass shrimp, and fishes have bioconcentrated Kepone from 425 to 20,000 times the concentration in the surrounding water. Therefore, action levels for edible seafoods now in force might be reached by as little as 5 parts of Kepone per trillion parts of water (nanograms per liter). In Kepone-free water, oysters can depurate about 90 percent of the accumulated Kepone in 4 days, but fish may require more than 3 weeks to lose 30 to 50 percent. Five weeks after fertilization of sheepshead minnow eggs containing Kepone, the juvenile fish retained as much as 46 percent of the Kepone present in the eggs. Kepone can be accumulated by fish to concentrations that exceed those in their food.

Kepone is acutely toxic to estuarine organisms, but long-term bioassays reveal that the hazard to these organisms is greatly underestimated by the 96-hour tests. The concentrations in micrograms per liter, estimated to be lethal to 50 percent of the test animals in 96 hours (LC_{50}), were 6.6 for spot, 70 for sheepshead minnows, 10 for an estuarine mysid, 121 for grass shrimp, and more than 210 for blue crabs. Kepone was lethal to adult sheepshead minnows exposed to 0.8 microgram per liter for 28 days. A significant number of embryos from adults exposed to 1.8 micrograms per liter were abnormal and died. When embryos were exposed to 0.08 microgram of Kepone per liter of water, 36 days later, resulting juvenile fish were shorter than control fish and some exhibited scoliosis. Mysid shrimp exposed for 20 days to about 0.2 microgram per liter produced fewer progeny; with greater concentrations, their growth and survival were reduced. We are concerned because all concentrations tested thus far in long-term exposures of sheepshead minnows and mysids have reduced survival, reproduction, or growth.

The threat of an even greater impact of Kepone to aquatic organisms in the James River and expansion of this impact into the Chesapeake Bay, therefore, is real and it may continue for some years to come. It is essential that we use knowledge now available to attempt to make decisions that may minimize the future impact of this insecticide on the aquatic environment.

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Mysidopsis bahia: An Estuarine Species Suitable for Life-Cycle Toxicity Tests to Determine the Effects of a Pollutant

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REFERENCE: Nimmo, D. R., Bahner, L. H., Rigby, R. A., Sheppard, J. M., and Wilson, A. J., Jr., "***Mysidopsis bahia:*** **An Estuarine Species Suitable for Life-Cycle Toxicity Tests to Determine the Effects of a Pollutant,**" *Aquatic Toxicology and Hazard Evaluation, ASTM STP 634,* F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, 1977, pp. 109–116.

ABSTRACT: This study documents the successful use of a mysid, *Mysidopsis bahia,* for life-cycle toxicity tests. These tests were conducted to determine acute and chronic toxicities of metal (cadmium) and pesticide (Kepone). Delay in the formation of mysid brood pouches and release of young were noted in low concentrations ≤ 6.4 μg cadmium/litre. Fewer young produced per female and decreased growth were other indicators of effects of Kepone.

KEY WORDS: water analysis, toxicology, toxicity, cadmium, Kepone, life cycles, insecticides

Many freshwater but a few estuarine or marine animals are available to the biologist for life-cycle toxicity tests. Few biologists have worked on methods of culturing marine animals; therefore, less is known about their nutritional, behavioral, or environmental requirements than those of freshwater species. Life cycles of marine species may be more complex, that is, many require an estuarine existence as larvae or juveniles, followed by adult migration to deeper waters offshore to reproduce. Culturing and maintaining estuarine and marine species require elaborate and expensive facilities because equipment must include temperature or salinity controls, anticorrosion surfaces, and often special filtration systems. Sufficient quantities of

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good quality saltwater may not be as available as freshwater and may be more expensive to obtain.

A water quality criteria report by the Environmental Protection Agency (EPA) [*1*][2] indicated that only 12 percent of 332 experiments with estuarine animals were conducted in flow-through tests with organic materials and that no tests were conducted on entire life cycles of any estuarine or marine species. Since this report, an entire life-cycle bioassay using the sheepshead minnow, *Cyprinodon variegatus,* has been accomplished [*2*].

We report progress in conducting life-cycle toxicity tests with small crustaceans called mysids. Commonly called an "opossum shrimp" because the female carries the young in a brood pouch during development, *Mysidopsis bahia* Molenock [*3*] was first described from West Bay, Galveston, Texas, and also occurs in southwest Florida [*4*]. Dr. T. E. Bowman[3] of the Smithsonian Institution, Washington, D.C., identified the species. We have completed tests on the effects of the metal, cadmium, and the pesticide, Kepone, which has been detected in the water, sediments, and biota of the James River and Chesapeake Bay [*5*]. The only other reported use of mysids for flow-through toxicity tests was to assay the toxicity of Kraft mill effluent [*6*].

Life History

Mysidopsis bahia is an estuarine species, but it has not been found at salinities below 9 parts per thousand salinity [*4*]. In our first collection, most animals were found in shallow ponds near the substratum, oriented positively towards the current. However, they made vertical migrations in early morning hours to feed at the surface. Scintila de Almeida Prado [*7*] found that six species of mysids closely related to *M. bahia* were primarily coastal-water species: the young migrate to surface waters at night, while adults remain close to the bottom. The vertical migration of the young facilitates their dispersion towards the sea or into adjacent mangrove areas. We have found that newly released *M. bahia* are planktonic for the first 24 h; thereafter, they orient to a current and actively pursue *Artemia.* Feeding habits of *M. bahia* have not been described. However, a related estuarine species, *M. almyra,* is omnivorous: 31 percent of its digestive tract contents is vascular plant detritus and 11 percent, copepods and diatoms [*4*].

Ecological Significance

The various stages in mysid life cycles are important links in estuarine and marine food chains, and many studies emphasize the importance of

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[2] The italic numbers in brackets refer to the list of references appended to this paper.

[3] Bowman, T. E., Smithsonian Institution, Washington, D.C., personal communication, 12 Jan. 1976.

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mysids as food for fishes [8–11]. Stomachs of young-of-the-year striped bass, *Morone saxatilis*, captured from the York River, Va., contained as much as 12.7 percent mysids by volume [11]. The composition of the stomach contents by volume of tidewater silversides, *Menidia beryllina*, included 65 percent mysids during night feeding [4]. Stickney et al [12] found that three of four species of flounders captured mysids as a primary staple in their diets. Percentage of total number of organisms in stomach contents were: *Etropus crossotus*, 3.1 percent; *Citharichthys spilopterus*, 72 percent; *Ancylopsetta quadrocellata*, 81 percent; and *Scopthalmus aquosus*, 96.3 percent. It is obvious that, in some instances, the loss of mysids as a food source would have serious impact on the next trophic level.

Materials and Methods

Mysids were collected from small shallow ponds that received a constant supply of saltwater from Santa Rosa Sound near Pensacola, Florida. Mysids were cultured in the laboratory in 40-litre glass aquaria supplied with filtered (20 μm) flowing water (10 to 27 parts per thousand salinity) at 18 to 28°C and were fed daily 48-h-old *Artemia salina* larvae. Overflow from each aquarium was through a standpipe to which a ring of screen (Nitex)[4] was attached to prevent escape of mysids and *Artemia*. This arrangement enabled us to culture the mysids continuously for 18 months without major fluctuations in population density.

All tests were conducted in intermittent flows from a diluter [13] or continuous flow with the toxicant added by an infusion pump [14]. As each aquarium achieved maximum volume, a self-starting siphon drained the water to a volume of about one litre in aquarium. Fluctuating levels, occurring at about 30 min each, ensured an exchange of water within each aquarium and the small chambers devised to retain the mysids. The chambers consisted of a standard glass petri dish to which a 15-cm-high cylinder of Nitex screen (mesh Number 210) was attached. To begin each test, twenty to thirty two 48-h-old juveniles, 4 or 5 per chamber, were exposed to each concentration. Acclimation was not necessary since the culture and test water were identical. A small stream of compressed air was delivered into each chamber to safeguard against possible anoxic conditions and to create a current that apparently aids orientation of the animals. In recording daily changes in populations, each chamber was lifted gently from the aquaria, water was drained through the Nitex cylinder to the level of the petri dish, and the chamber was then placed on a lighted counter top. Number of live animals by sex, number of females with and without brood pouches, and number of young were recorded.

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[4] Nitex is a registered trademark of Tobler, Ernst and Trabor, Inc., Murray St., New York, N.Y. Reference to commercial products does not constitute endorsement by the Environmental Protection Agency.

We conducted 96-h, flow-through tests beginning with 48-h-old juvenile mysids, followed by life-cycle bioassays. The procedures were similar, except that test concentrations in the life-cycle tests were lower than in the 96-h tests.

Water samples were analyzed for Kepone by extracting one litre of seawater twice with 100-ml volumes of methylene chloride in a two-litre separatory funnel. The combined extracts were concentrated to about 5 ml in a Kuderna-Danish Concentrator on a steam table. Fifteen millilitres of benzene was added and the extract reconcentrated to remove the methylene chloride. Extracts were adjusted to appropriate volumes for analyses by electron-capture gas chromatography. Determinations were performed on Varian Aerograph Model 2100, and 1400 gas chromatographs equipped with 182-cm by 2-mm inside diameter glass columns packed with 2 percent SP 2100 and 0.75 percent SP2250: 0.97 percent SP2401 on 100/120 mesh Supeloport.

The operating modes were: oven temperature, 185°C; injector temperature, 200°C; detector temperature, 210°C; nitrogen carrier-gas flow rate, 25 ml/min. Average recovery of Kepone from saltwater was 85 percent; concentrations reported here were not corrected for efficiency of the analytical method. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of results.

Cadmium in water was measured by atomic absorption spectrophotometry, by using the procedure of Nimmo et al [*15*].

The LC_{50}s and 95 percent fiducial limits were calculated by linear regression analysis after probit transformation. We employed two-sample *t* test to mean brood size; we used Dunnett's test comparing multiple treatments with control to mean adult length. Significant ($\alpha = 0.05$) differences were attributed to Kepone.

Results and Discussion

Life-cycle toxicity tests, using survival as the criterion for effects, were first conducted with cadmium; the procedures for quantifying effects or the reproduction and growth were developed while testing the pesticide, Kepone. Seventeen days was chosen as the test requirement for a life cycle because animals in the control aquarium released their brood in this time. In subsequent experiments, we reduced the time required to complete a life cycle by maintaining the temperature at 29°C.

For cadmium, the 96-h calculated LC_{50} was 15.5 μg/litre (95 percent fiducial interval, 12.6 to 19.6) at 25 to 28°C and 10 to 17 parts per thousand salinity. In a 17-day life-cycle test, the LC_{50} was 11.3 μg/litre (95 percent fiducial interval, 4.2 to 12.9 at 20 to 28°C and 15 to 23 parts per thousand salinity (Table 1)). In addition, we observed a 48-h delay in the formation of brood pouches and a 24-h delay in the release of brood in

TABLE 1—*Survival (numbers) of* Mysidopsis bahia *in measured concentrations of cadmium chloride in seawater. Temperature range, 20 to 28°C; salinity, 15 to 23 parts per thousand.*

Days	Cadmium, µg/litre				
	Control	4.8	6.4	10.6	28.0
0	20	20	21	20	20
11	19[a]	19[a]	16	16[a]	1
13	19	19	16[a]	16	0
17	19[b]	19[b]	16	13	0
18	19	19	16[b]	11	0
20	19	19	16	8[b]	0
23	19	19	16	2	0

[a] Formation of brood pouches noted in the chambers.

[b] Young released. Average number of young/female at 23 days was 7.0 in control, 8 in 4.8 µg/litre, 3 in 6.4 µg/litre, and 4 in 10.6 µg/litre. This average was based on all females, not producing females alone.

females exposed to 6.4 µg/litre. Although we were unable to prove statistical significance of these observations, we believe that these delays, and a 72-h delay in release of brood by females in the 10.6 µg/litre, could have a deleterious effect ecologically since production of maximum brood is out of phase with food or predator cycles. Also, fewer young were produced by females in the 6.4 and 10.6 µg/litre aquaria than by females in the control and 4.8 µg/litre aquaria.

The 96-h calculated LC_{50} for Kepone toxicity to *M. bahia* exposed to Kepone was 10.1 µg/litre (95 percent fiducial interval, 8.1 to 12.4) at 25 to 28°C and 10 to 16 parts per thousand salinity. The 19-day (life-cycle) LC_{50} was 1.4 µg/litre (95 percent fiducial interval, 1.1 to 1.8) at 25 to 28°C and 10 to 20 parts per thousand salinity (Table 2). The duration of the test allowed production of several broods. We discovered that a single female could commence brood production as early as Day 12 at higher temperatures, and could produce two additional broods by Day 20; some females produced as many as 35 young. In the Kepone test, the average number of young per female at 20 days was 15.3 in the controls, and 8.9 in 0.39 µg/litre. Differences between controls (15.3 juveniles/female) and the 0.39 µg/litre concentration (8.9 juveniles/female) were significant (α = 0.05, 2-sample *t* test).

In preliminary tests, growth of some mysid individuals in higher concentrations of Kepone appeared to be less than control mysids. To evaluate this effect, we began two separate, 14-day tests that began by exposing 24-h-old juveniles to Kepone and concluded by measuring their total lengths (tip of carapace to end of uropod) (Table 3). We found that female mysids exposed to 0.072 µg/litre Kepone grew less than the control mysids. This effect was consistent with apparent effects on reproductive success

TABLE 2—*Survival (numbers) of* Mysidopsis bahia *in measured concentrations of Kepone in seawater. Temperature was 25 to 28°C; salinity, 10 to 20 parts per thousand.*

	Kepone, μg/litre				
Days	Control	0.39	1.55	4.4	8.7
0	32	32	32	32	32
2	32	32	26	19	5
4	32	32	22	9	0
6	32	29	21	6	0
8	32	29	21	2	0
10	31	29	19	2	0
15	30	27	17	1	0
19	29	27	16	1	0

NOTE—Average number of young per producing female at 19 days was 15.3 in control, 8.9 in 0.39 μg/litre, and 0 in all higher concentrations tested. The difference between control (15.3) and 0.39 μg/litre (8.9) was significant (2-sample *t* test).

TABLE 3—*Lengths of 14-day mysids,* Mysidopsis bahia, *maintained in sublethal measured concentrations of Kepone. Measurements were from tip of carapace to end of uropod. Temperature, 24 to 29°C; salinity, 18 to 27 parts per thousand.*

	Kepone, μg/litre					
Average Length, mm	Control	0.026	0.072	0.11	0.23	0.41
Males	7.96	7.88	7.70	7.93	7.67	7.71
Females	8.32	8.34	8.11[a]	8.16[a]	8.33	8.06[a]

[a] Significant at 95% confidence level compared to control (Dunnett's test).

(fewer juveniles/female). Tests with 13 species of malacostracans (including 5 species of mysids) have established that the number of eggs produced was a direct function of body length [*16*].

Our toxicity data for mysids exposed to cadmium were similar to those reported for other species. For example, after 96 h, the estimated LC_{50} for sand shrimp, *Crangon septemspinosa,* and hermit crabs, *Pagurus longicarpus,* to cadmium was 320 μg/litre [*17*]. By comparison, the 30-day LC_{50} value for pink shrimp, *Penaeus duorarum,* was 720 μg/litre, and a 29-day LC_{50} for grass shrimp, *Palaemonetes vulgaris,* was 120 μg/litre [*15*]. Thus, in either 96-h or life-cycle bioassays, the sensitivity of *M. bahia* to cadmium was an order of magnitude greater than that of the four species of decapod crustaceans just reported. In addition, crustaceans were found to be more sensitive to cadmium than were echinoderms, annelids, mollusks, or fishes [*17*].

Mysids were sensitive to Kepone in 96 h (10.1 µg/litre) [*5*]. The 96-hour LC_{50} for several other estuarine organisms were: 6.6 µg/litre for spot (*Leiostomus xanthurus*),70 µg/litre for sheepshead minnows (*Cyprinodon variegatus*), 121 µg/litre for grass shrimp (*Palaemonetes pugio*), and more than 210 µg/litre for blue crabs (*Callinectes sapidus*).

Our data for cadmium and Kepone provide bases for calculation of application factors. This factor is the numerical ratio of the "safe" concentration (conducted in a long-term toxicity test) to the acutely lethal concentration (usually a 96-h toxicity test) [*18*]. Although application factors have been used primarily for fishes, sufficient data are lacking to allow such estimates for invertebrates or for extrapolating between phylogenetic lines, especially for marine species for which few data exist. However, by using the procedure given by Eaton [*18*], the application factor for cadmium can be calculated by dividing the concentration that did not delay production of young mysids nor decrease number of young per female (4.8 µg/litre) by the 96-h LC_{50} (15.5 µg/litre). The resulting ratio is 0.31. The application factor for Kepone can be calculated by dividing the concentration that had no apparent effect on growth of female mysids (0.026 µg/litre) by the 96-h LC_{50} (10.1 µg/litre). This ratio is 0.0025. Since both cadmium and Kepone are considered persistent and cumulative, the water quality criteria report [*1*] has recommended that concentrations of these chemicals should not exceed 0.05 of the 96-h LC_{50} at any time or place. In addition, the 24-h average concentration should not exceed 0.01 of the 96-h LC_{50}. Our research with mysids suggests that extreme caution should be taken when estimating application factors (for example, 0.01) without data from laboratory studies, because the laboratory-derived application factor is "overprotective" for cadmium (0.31) and "underprotective" for Kepone (0.0025).

General Conclusions

Ease of culture and maintenance in flow-through bioassays, apparent sensitivity to toxicants, and shortness of life cycle are advantages for using the crustacean *Mysidopsis bahia* in life-cycle toxicity tests. Some sublethal effects observed after prolonged exposure to cadmium and Kepone singly were: (*a*) delay in the formation of brood pouches, (*b*) delay in the release of young, (*c*) fewer young produced per female, and (*d*) reduced growth. In nature, the loss of mysids due to the direct toxic effects of pollutants or the indirect effects on their growth or population size could affect the food supply of many fishes.

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Short Papers and Notes

Toxicity and Uptake of Kepone in Marine Unicellular Algae[1]

ABSTRACT: Four species of marine unicellular algae were exposed to Kepone in laboratory bioassays. EC50 values after seven days' growth, in mg/liter (ppm), were: *Chlorococcum* sp., 0.35; *Dunaliella tertiolecta*, 0.58; *Nitzschia* sp., 0.60; *Thalassiosira pseudonana*, 0.60. When exposed to 100 μg/liter (ppb) Kepone for 24 hr, residues associated with the algae, in mg/kg (ppm) wet weight, were: *Chlorococcum* sp., 80; *D. tertiolecta*, 23; *Nitzschia* sp., 41; *T. pseudonana*, 52.

Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene-2-one) is used for control of banana and potato pests and as an ant and roach killer. The insecticide has been reported in water and biota of the Appomatox and James rivers, Virginia (Environmental Protection Agency 1975). Kepone is structurally similar to the insecticide mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene) and may be a product of its photodegradation (Ivie et al. 1974).

We exposed four species of marine unicellular algae to Kepone to determine its effects on growth and its uptake by phytoplankton. Since algae are food for animals, oxygenate water, and regulate nutrients, an effect on them could affect physical, chemical, and biological processes.

Materials and Methods

Algae tested were *Chlorococcum* sp. (Milford "C"), *Dunaliella tertiolecta* (chlorophytes), *Nitzschia* sp. (LB 684), and *Thalassiosira pseudonana* (bacillariophytes). All were obtained from the Culture Collection of Algae, Indiana University.

In growth studies, algae were grown either in: (1) 25 ml of medium in optically matched test tubes shaken at approximately 60 excursions per minute on a New Brunswick Model G shaker fitted with an ErlAngle® (New Brunswick Scientific Co., New Brunswick, Connecticut) clamp, or (2) 50 ml of medium in 125-ml Erlenmeyer flasks shaken at approximately 60 excursions per minute. In the former, optical density at 525 nm on a Fisher electrophotometer was determined each day for seven days after inoculation; in the latter, optical density was determined after seven days of growth.

Growth medium was artificial seawater of 30 parts per thousand salinity with full and half-nutrient strengths (Hollister et al. 1975). Temperature was 20° ± 0.5°C under 5,000 lux illumination from cool, white fluorscent tubes with alternate 12-hr periods of light and darkness. Technical grade Kepone was added in 0.1 ml acetone and 0.1 ml acetone was added to all control cultures. This concentration of acetone did not affect growth. During seven growth tests, each exposure was performed three times. Concentrations that reduced growth by 50 per cent (EC50) after seven days were calculated (American Public Health Association 1971).

In uptake studies, 12 cultures of each alga were grown in 100 ml of full nutrient medium for six days. All that time, cell numbers were between 2×10^5 and 3×10^5 cells/ml, and Kepone, in 0.1 ml acetone, was then added at the nominal concentration of 100 μg/liter. This was well below its solubility limit of 2–4 mg/liter in seawater (May, unpublished). On the seventh day, the algae were harvested by centrifugation, washed four times with uncontaminated medium, and analyzed for kepone by gas chromatography. Each value in Table 1 is the average of 12 replications.

For residue analysis, algae samples were weighed in 125 mm × 15 mm (O.D.) screw-top test tubes and extracted with two 5-ml portions of acetonitrile for 30 sec with a Model PT 10-ST Willems Polytron (Brinkmann Instruments, Westbury, New York). The acetonitrile extract was transferred to a 250-ml separatory funnel after centrifugation, and the algae again extracted, first with 5 ml and then with 10 ml of acetone. After each extraction, the tube was centrifuged, and the extract placed in the separatory funnel. Then, 100 ml of 2.0% aqueous sodium sulfate and 10 ml of 1:1 diethyl ether-petroleum ether was added, and the mixture shaken for 1 minute. After the solvent phases separated, the lower aqueous phase was drained into a 250-ml beaker and the upper layer collected in a 25-ml Kuderna-Danish concentrator tube. The ether extraction was repeated three times with 5 ml of 1:1 diethyl ether-petroleum ether. The combined extracts were concentrated to dryness by placing the tube in a water bath at 45°C and evaporating the solvent with a gentle stream of nitrogen. The residue was transferred to a 200 mm × 9 mm (I.D.) Chromaflex® column (Kontes Glass Co, Vineland, N.J.) containing 3.0 gm of Florisil® overlain by 2.0 gm of anhydrous sodium sulfate. The column was first washed with 10 ml of hexane and the residue transferred with four 0.5-ml volumes of 5% diethyl ether in hexane to remove PCB and pesticides. Kepone was removed by a second elution with 40 ml of 1% methanol in benzene. Extracts concentrated or diluted to appropriate volume were analyzed by electron-capture gas chromatography on Varian Aerograph Models 2100 and 1400 Gas Chromatographs equipped with 182 cm × 2 mm (I.D.) glass columns packed with 2% SP2100 and 0.75% SP2250:0.97%

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[1] Contribution No. 292 from the Environmental Research Laboratory - Gulf Breeze.

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TABLE 1. Depression of growth of marine unicellular alga by Kepone and concentrations of Kepone in algae after exposure to 100 μg/l for 24 hours.

Algae	EC50[a] μg/l	Cell Residue μg/g	Concentration Factor
Chlorococcum sp.	0.35 (0.27–0.40)	80	800
D. tertiolecta	0.58 (0.51–0.64)	23	230
Nitzschia sp.	0.60 (0.53–0.66)	41	410
T. pseudonana	0.60 (0.50–0.70)	52	520

[a] Range in parentheses.

SP2401 on 100/120 mesh Supelcoport®. The operation conditions were: oven temperature, 185°C; injector temperature, 200°C; detector temperature, 215°C; nitrogen carrier gas flow rate, 25 ml/min. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of results. The average recovery rate of Kepone from fortified algae was 85%. Residue concentrations were calculated on a wet weight basis without correction factor for percentage recovery.

Results

Effects and uptake of Kepone are given in Table 1. Because results from bioassays with full and half-strength nutrient media were identical, the data were combined. Seventh-day EC50 values are representative of effect because the degree of growth inhibition was constant from day to day. *Chlorococcum* sp. was the most sensitive species: its EC50 values never overlapped those of the others. The EC50 values of the other species were nearly identical.

Chlorococcum sp. also accumulated the greatest amount of Kepone from the medium in the 24-hr exposure, and all differences, except between *Nitzschia* sp. *and T. pseudonana*, are highly significant statistically (t-test for two means, Brownlee 1965).

Discussion

Butler (1963) stated that 1 mg/liter of Kepone reduced carbon fixation by estuarine phytoplankton by 95 per cent after exposure for 4 hr. Our data show that toxicity occurred at much lower concentrations and that algae can accumulate the chemical from water. Since EC50 concentrations were higher than those reported from the Appomatox and James rivers (Environmental Protection Agency 1975), Kepone may have little effect on phytoplankton in the field. However, uptake by algae could result in accumulation at higher trophic levels.

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Acute Toxicity of Kepone® to Four Estuarine Animals[1]

ABSTRACT: Recent contamination of the James River estuary, Virginia, with Kepone prompted acute flow-through bioassays to determine the 96-hour toxicity of the insecticide to four estuarine species native to that ecosystem. The species and their 96-hour LC50 values were: grass shrimp (*Palaemonetes pugio*), 121 μg/liter; blue crab (*Callinectes sapidus*), >210 μg/liter; sheepshead minnow (*Cyprinodon variegatus*), 69.5 μg/liter; and spot (*Leiostomus xanthurus*) 6.6 μg/liter. Surviving animals were analyzed for Kepone. Average bioconcentration factors (the concentration of Kepone in tissues divided by the concentration of Kepone measured in seawater) were: grass shrimp, 698; blue crab 8.1; sheepshead minnow, 1,548; and spot, 1,221.

Introduction

Few published data are available on Kepone toxicity to estuarine animals. Butler (1963) reported EC50 values (based on mortality or loss of equilibrium in 48 hours for shrimp and on inhibition of shell deposition in 96 hours for oysters) of 85 μg/liter for brown shrimp (*Penaeus aztecus*); 57 μg/liter and 15 μg/liter for eastern oysters (*Crassostrea virginica*) exposed at seawater temperatures of 14°C and 31°C, respectively. Twenty percent of the blue crabs (*Callinectes sapidus*) exposed to 1,000 μg/liter Kepone died in 48 hours. Butler's data were derived from flow-through bioassays and based on nominal, not measured, concentrations of Kepone in seawater.

Recent discharge of the insecticide, Kepone®, into the James River estuary, Virginia has resulted in contamination of that system's water, sediment, and biota. This contamination raised questions about the acute and chronic effects of Kepone on the aquatic life in the estuary and the potential danger to humans by eating contaminated animals.

In January 1976, we initiated flow-through bioassays to determine bioconcentration and acute toxic effects of Kepone on representative species found in the James River estuary. These were grass shrimp (*Palaemonetes pugio*), blue crab (*Callinectes sapidus*), sheepshead minnow (*Cyprinodon variegatus*), and spot (*Leiostomus xanthurus*).

Methods and Materials

Acute toxicity was determined by exposing 20 animals per aquarium to different concentrations of Kepone for 96 hours in flow-through bioassays similar to those described by Lowe et al. (1972). All test animals were acclimated to laboratory conditions for at least ten days prior to testing. The temperature and salinity of seawater in which they were held were allowed to vary with those of Santa Rosa Sound, Florida, during acclimation and testing. Our acclimation and testing procedures were compatible with those of Standard Methods (A.P.H.A. 1971). All test animals were captured in the vicinity of the Gulf Breeze Laboratory and samples contained no detectable Kepone (<0.02 μg/g). During acclimation, test animals were fed frozen brine shrimp daily. Animals were not fed during the tests, but could obtain food (plankton and other particulate matter) from the unfiltered seawater. Seawater was pumped from Santa Rosa Sound into a constant-head trough in the laboratory and delivered to each 18 liter aquarium by a calibrated siphon that delivered approximately 68 liter/hr. One control and five experimental aquaria were used in each test. Stock solutions of Kepone (88% pure), in reagent-grade acetone, were metered into experimental aquaria at the rate of 60 ml/hr.

The 96-hour LC50 values were determined for both nominal and measured concentrations of Kepone in seawater. Nominal concentrations were those calculated to be in seawater, based on the concentration of the stock solution, plus the stock solution and seawater flow rates. The LC50 values were based on measured Kepone concentrations determined by chemical analysis of the exposure water. Mortality data were subjected to probit analysis to determine LC50 values and their 95% confidence limits (Finney 1971).

At the end of each 96-hour test, surviving animals from each concentration were sacrificed, rinsed with acetone, and pooled as a single sample for residue analysis.

Water samples were analyzed by [illegible] of seawater twice with 100 ml of methylene chloride. The combined extracts were concentrated to about 5 ml in a Kuderna-Danish Concentrator on a steam table. Fifteen milliliters of benzene were added and the extract reconcentrated to remove the methylene chloride. The extract was cleaned up on a Florisil Column as described below.

Tissues of shrimp, crabs, or fish were weighed in 150 mm × 25 mm (O.D.) screw-top test tubes and extracted twice with 5 ml volumes of acetonitrile for 30 seconds with a model PT 10-ST Willems Polytron (Brinkman Instruments, Westbury, New York). The mixture was centifuged and the acetonitrile transferred to a 250-ml separatory funnel. After the second extraction, the tissue was extracted with one 5-ml and one 10-ml volume of acetone. After each acetone extraction the tube was centrifuged and the acetone added to the 250-ml separatory funnel. To the combined extracts, 100 ml of 2.0% aqueous sodium sulfate and 10 ml of 1:1 diethyl ether-petroleum ether were added. The separatory funnel was shaken for one minute. After the solvent phases had separated, the lower aqueous phase

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[1] Contribution No. 293, Environmental Research Laboratory, Gulf Breeze.

TABLE 1. Toxicity of Kepone to and uptake by four estuarine organisms after 96 hours exposure.

Species	Water Concentration (μg/liter) Nominal	Water Concentration (μg/liter) Measured	Mortality (%)	Whole-Body Residue (μg/g wet weight)	Bioconcentration Factor
Grass shrimp (*Palaemonetes pugio*)	Control	ND[a]	0	ND[a]	–
	13.5	12.	0	5.1	425
	24.	15.	0	14.	933
	42.	39.	0	29.	744
	75.	69.	5	42.	609
	135.	121.	50	94.	777
					$\bar{x}$ = 698
Blue crab (*Callinectes sapidus*)	Control	ND	0	ND	–
	42.	–	0	–	–
	75.	–	10	–	–
	135.	110.	5	0.85	7.7
	187.	164.	0	1.7	10.4
	240.	210.	0	1.3	6.2
					$\bar{x}$ = 8.1
Sheepshead minnow (*Cyprinodon variegatus*)	Control	ND	0	ND	–
	10.	7.1	0	11.2	1577
	18.	14.	0	20.9	1493
	32.	23.	5	44.4	1930
	56.	51.5	20	63.6	1235
	100.	78.5	65	118.4	1506
					$\bar{x}$ = 1548
Spot (*Leiostomus xanthurus*)	Control	ND	0	ND	–
	2.4	1.5	5	1.7	1133
	4.2	3.4	10	3.2	941
	7.5	4.4	45	7.0	1591
	13.5	7.8	40	10.8	1385
	24.	15.9	95	16.8	1057
					$\bar{x}$ = 1221

[a] ND – non detectable; <0.02 μg/liter in water, <0.02 μg/g in tissue.

TABLE 2. 96-hour toxicity of Kepone to several estuarine animals in flowing seawater bioassays. The 95% confidence intervals are in parentheses. Animal sizes are rostrum-telson length for shrimps, carapace width for crabs, and standard length for fishes.

Species	Size ($\bar{x}$, mm)	Nominal 96-hour LC50 in μg/liter (95% Confidence Limit)	Measured 96-hour LC50 in μg/liter (95% Confidence Limit)	Temperature ($\bar{x}$, °C)	Salinity ($\bar{x}$, ‰)
Grass shrimp (*Palaemonetes pugio*)	27.8	134.8 (114.1-193.9)	120.9 (103.0-171.6)	20.0	16.0
Blue crab (*Callinectes sapidus*)	34.3	>240	>210	19.0	20.0
Sheepshead minnow (*Cyprinodon variegatus*)	20.0	83.0 (67.9-115)	69.5 (56.3-99.5)	18.0	15.0
Spot (*Leiostomus xanthurus*)	33.9	10.5 (8.3-14.0)	6.6 (5.3-8.8)	25.0	18.0

was drained into a 250-ml beaker and the upper ether layer was collected in a 25-ml Kuderna-Danish concentrator tube. The ether extraction was repeated three times with 5 ml of 1:1 diethyl ether petroleum ether. The combined extracts were concentrated just to dryness by placing the concentrator tube in a water bath at 45°C and blowing off the solvent with a gentle stream of nitrogen. The residue was transferred to a 200 mm × 9 mm (I.D.) Chromaflex column (Kontes Glass Co.) containing 2.3 gm of Florisil topped with 2.0 gm of anhydrous sodium sulfate. The column initially was washed with 10 ml of hexane and the residue transferred with four 0.5 ml volumes of 5% diethyl ether in hexane. The column was eluted with 20 ml of 5% diethyl ether in hexane to remove PCB and pesticides. Kepone was eluted in a second elution of 40 ml of 1% methanol in benzene. Extracts were concentrated or diluted to appropriate volumes for analyses by electron capture gas chromatography.

Determinations were obtained by Varian Aerograph Model 2100 and 1400 Gas Chromatographs equipped with 182 cm × 2 mm (I.D.), glass columns packed with 2% SP2100 and 9.75% SP2250: 9.97% SP2401 on 100/120 mesh Supelcoport. The operating parameters were: oven temperature 185°C, injector temperature 200°C, detector temperature 216°C, and nitrogen carrier gas flow rate 25 ml/min.

The average recovery rate of Kepone from fortified tissue was 87%; from water, 85%. Residue concentrations were calculated on a wet-weight basis without a correction factor for percentage recovery. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of the results.

Results and Discussion

Kepone, at concentrations tested, was acutely toxic to shrimp and fishes but not to blue crabs. The LC50 values varied widely among species. Spot were the most sensitive with a 96-hour LC50 of 6.6 μg/liter; the sheepshead minnow LC50 was over ten times higher (69.5 μg/liter). The two crustaceans were less sensitive. Grass shrimp LC50 was 120.9 μg/liter, and no significant mortality was observed in blue crabs at measured concentrations as high as 210 μg/liter (Tables 1 and 2).

Although the sensitivity of fish to Kepone toxicity differed, the symptoms of Kepone poisoning were similar. An early symptom was lethargic behavior followed by loss of equilibrium. These symptoms occurred in sheepshead minnows at 48 hours in 56 and 100 μg/liter concentrations and 96 hours in 18 and 32 μg/liter concentrations. Spot exhibited the symptoms in 48 hours when exposed to 7.5, 13.5 and 24 μg/liter Kepone. An advanced stage of poisoning was evident in dark coloration of portions of the fish's body. This color change was striking in that some fish had normal coloration on one side of their bodies, while the other side was nearly black with a sharp line of demarcation. Some spot and sheepshead minnows were darkened in only one quadrant of the body; for example, the left side posterior to the pectoral fins. These color changes were always more marked and appeared earlier in the higher Kepone concentrations. Hansen et al. (1977) also noted color changes in sheepshead minnows exposed to a lower Kepone concentration (0.8 μg/liter) over a longer duration (11 days). The same authors also noted that growth, reproduction and survival of sheepshead minnows were affected in 36 days by Kepone concentrations as low as 0.08 μg/liter, which is 0.001 of our sheepshead minnow LC50 (69.5 μg/liter). If we assume that the same ratio exists for spot as Hansen et al. reported for sheepshead minnows, then the no-effect level for spot would be less than 0.007 μg/liter based on our spot LC50 (6.6 μg/liter).

No color changes were observed in the two crustaceans although they also were lethargic in Kepone concentrations greater than 75 μg/liter.

Kepone was bioconcentrated by all test animals in 96 hours, although bioconcentration factors (concentration of Kepone in tissue divided by measured Kepone in water) varied between species (Table 1). (The bioconcentration factors for fish were similar, $\bar{x}$ = 1200–1500). The two fishes bioconcentrated Kepone an average of 1.7 to 2.2 times the bioconcentration factor for the grass shrimp and 150 to 190 times that measured in the blue crab. This difference in bioconcentration has been noted in similar bioassays with other organochlorine insecticides. Schimmel et al. (1976) reported that sheepshead minnows, spot, and pinfish (*Lagodon rhomboides*) bioconcentrated 4 to 70 times more heptachlor than grass shrimp or pink shrimp (*Penaeus duorarum*). A similar relationship occurred when some of the same species were exposed to toxaphene (Schimmel et al., in press) and dieldrin (Parrish et al. 1974) in 96-hour bioassays. The reason for an extremely low bioconcentration factor in the blue crab compared to grass shrimp is not known.

Further studies over a longer period of time are required to better understand the more subtle effects of Kepone on estuarine animals. One reason for this assessment is that most deaths occurred after 48 hours in our tests. These studies should include: (1) long-term bioconcentration studies; (2) bioassays which include the hatching and early development of an estuarine animal such as spot; and (3) studies to determine movement of Kepone through an estuarine food web.

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Kepone®: Chronic Effects on Embryo, Fry, Juvenile, and Adult Sheepshead Minnows (*Cyprinodon variegatus*)[1]

ABSTRACT: We investigated the toxicity of Kepone to, and uptake by embryo, fry, juvenile, and adult sheepshead minnows (*Cyprinodon variegatus*) using intermittent-flow toxicity tests. Concentrations of Kepone and percentage of adult fish surviving in a 28-day exposure were: Control, 95%; 0.05 µg/liter, 95%; 0.16 µg/liter, 100%; 0.80 µg/liter, 78%; 1.9 µg/liter, 20%; and 7.8 µg/liter and 24 µg/liter, 0%. Concentration factors (concentrations in fish divided by concentrations measured in water) for adult fish averaged 5,200 (range 3,100 to 7,000). Symptoms of poisoning included scoliosis, darkening of the posterior one-third of the body, hemorrhaging near the brain and on the body, edema, fin-rot, uncoordinated swimming, and cessation of feeding. Adults surviving the first exposure [illegible] ing, and survival and growth of fry and juveniles were monitored in a 36-day exposure to Kepone concentrations of 0.08, 0.18, 0.72, 2.0, 6.6, and 33 µg/liter. A significant number of embryos from adult fish exposed to 1.9 µg of Kepone/liter of water developed abnormally and died even when incubated in Kepone-free water. Kepone in water was not as lethal to progeny as to adults: 36-day LC50 for juveniles was 6.7 µg/liter; 28-day LC50 for adults, 1.3 µg/liter. However, the average standard length of juvenile fish was significantly reduced by exposure to 0.08 µg of Kepone/liter of water; some fish developed scoliosis. Concentration factors in juvenile sheepshead minnows averaged 7,200 and increased from 3,600 to 20,000 as exposure concentrations decreased.

Introduction

Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta cd pentalene-2-one, has been found recently in estuarine organisms in the James River, Virginia (Hansen et al. 1976). The acute toxicity of this insecticide to estuarine fishes and invertebrates has been investigated (Butler 1963; Schimmel and Wilson 1977), but the chronic effects of Kepone on estuarine fish have not.

Our study was conducted to determine: 1) the effects of Kepone exposure on survival and reproduction of adult sheepshead minnows, 2) the effect on survival and growth of embryos, fry, and juvenile fish spawned from exposed adults, and 3) the extent of bioconcentration in adults and progeny.

Materials and Methods

TEST ANIMALS

Adult sheepshead minnows were [illegible] ected near the Environmental Research Laboratory, Gulf Breeze, Florida and acclimated in test aquaria in 30°C water for 15 days before exposure. Mortality during acclimation was less than 1 percent, and the fish fed, spawned, and otherwise behaved normally. Tested fish averaged 40 mm standard length and 2.3 g. Adult fish and their food, Biorell® and frozen adult brine shrimp (*Artemia salina*), contained no detectable (< 0.02 µg/g) Kepone, other chlorinated insecticides or PCB's.

ADULT EXPOSURE

We exposed 32 female and 32 male fish in aquaria containing zero, 0.16, 0.80, 1.9, 7.8, and 24 µg/liter of Kepone, and 108 fish to 0.05 µg/liter for four weeks in an intermittent-flow toxicity test. Our apparatus was a modified model of that used by Schimmel et al. (1974). We delivered Kepone, 0.0088 ml of the solvent triethylene glycol, and 1.5 liter of filtered 30°C (± 1°C) seawater averaging 15‰ salinit (range 8-26 ‰) to each 70 liter aquarium during each cycle of the apparatus. Water and solvent without Kepone were delivered to the control aquarium. Number of cycles per day for the adult exposure averaged 440.

EMBRYO, FRY, AND JUVENILE EXPOSURE

To determine the effect of Kepone on sheepshead minnow embryo, fry, and juvenile survival, we en-

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® Registered trademark, Allied Chemical Corporation, 40 Rector Street, New York, New York 10006. Kepone used was purchased from Chem Service, West Chester, PA as 99% pure. Our analyses indicated 88% purity. Mention of trade names or commercial products does not constitute endorsement by the Environmental Protection Agency.

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hanced egg production by injecting hormones in exposed adult fish, fertilizing the eggs artifically, and monitoring their development in Kepone for 36 days. Twenty female sheepshead minnows exposed to none, 0.05, 0.16, or 0.80 μg/liter of Kepone and 8 females exposed to 1.9 μg/liter were injected with 50 I. U. of human chorionic gonadotrophic hormone on exposure days 25 and 27 (Hansen et al. 1974). On the 28th day of exposure eggs were stripped manually from injected fish and were fertilized in control water with sperm from excised macerated testes from ten or more males exposed to 0.8 μg/liter or less of Kepone and from five fish exposed to 1.9 μg/liter. Some spawned adult fish from each concentration were frozen for chemical analyses of Kepone content.

Embryos were placed in appropriate aquaria previously used for adult fish to determine the effects of Kepone 1) in water; 2) within the egg; and 3) in both eggs and water on embryos, fry, and juvenile fish. Twenty embryos from control fish were placed in each of four egg cups (Petri dishes to which a nine-cm high collar of 450μ nylon mesh was attached) in the control aquarium and in each aquarium receiving Kepone. Twenty embryos from fish in each aquarium receiving Kepone were placed in each of four egg cups in that aquarium and into four egg cups in the control aquarium. Thus a total of 80 embryos was observed in each of 15 separate treatments. Remaining eggs were frozen for chemical analysis of Kepone content. The dosing apparatus cycled about 350 times per day delivering water to each aquarium containing egg cups. The action of a self-starting siphon in each aquarium caused water levels to fluctuate approximately 5 cm about 40 times per day insuring that water in the egg cups was exchanged. Fry were fed live brine shrimp nauplii that contained no detectable (<0.02 μg/g) Kepone, other chlorinated insecticides, or PCB's. At the end of the 36-day embryo-fry-juvenile exposure, juveniles were photographed for length measurements, weighed, and some were frozen for chemical analyses.

Chemical Analyses

Water samples were analyzed by extracting one liter of seawater twice with 100 ml of methylene chloride. The combined extracts were concentrated to approximately 5 ml in a Kuderna-Danish Concentrator on a steam table, and 15 ml of benzene was added. The extract was reconcentrated to remove the methylene chloride and was cleaned on a Florisil Column (described below).

Tissues of fish and fish eggs were weighed in 150 mm × 25 mm (O.D.) screw-top test tubes and were extracted twice with 5-ml volumes of acetonitrile for 30 seconds with a model PT 10-ST Willems Polytron (Brinkman Instruments, Westbury, New York). The test tube was centrifuged and the acetonitrile was transferred to a 250-ml separatory funnel. After the second extraction, the tissue was extracted with one 5-ml and one 10-ml volume of acetone. After each acetone extraction, the tube was centrifuged and the acetone was added to the 250-ml separatory funnel. To the combined extracts were added 100 ml of 2.0% aqueous sodium sulfate and 10 ml of 1:1 diethyl ether-petroleum ether. The separatory funnel was shaken for one minute. After the solvent phases separated, the lower aqueous phase was drained into a 250-ml beaker and the upper ether layer was collected in a 25-ml Kuderna-Danish concentrator tube. The ether extraction was repeated three times with 5 ml of 1:1 diethyl ether-petroleum ether. The combined extracts were concentrated to just dryness by placing the concentrator tube in a water bath at 45°C and blowing off the solvent with a gentle stream of nitrogen. The residue was transferred to a 200 mm × 9 mm (I.D.) Chromaflex column (Kontes Glass Co.) containing 3.0 gm of Florisil topped with 2.0 gm of anhydrous sodium sulfate. The column was washed initially with 10 ml of hexane and the residue was transferred with four 0.5 ml volumes of 5% diethyl ether in hexane. PCB's and pesticides were eluted from the column with 20 ml of 5% diethyl ether in hexane. Then, Kepone was eluted in 40 ml of 1% methanol in benzene. Individual extracts were concentrated or diluted to appropriate volumes for analyses by electron-capture gas chromatography.

Determinations were performed on Varian Aerograph Model 2100 and 1400 Gas Chromatographs equipped with 182 cm × 2 mm (I.D.) glass columns packed with 2% SP2100 and 0.75% SP2250: 0.97% SP2401 on 100/120 mesh Supelcoport. The operating conditions were: oven temperature, 185°C; injector temperature, 200°C; detector temperature, 210°C; nitrogen carrier-gas flow rate, 25 ml/minute.

The average recovery rate of Kepone from fortified tissue was 87% and from water, 85%. Residue concentrations were calculated on a wet-weight basis without a correction factor for percentage recovery. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of the results.

Statistical Analyses

Probit analyses of mortality data were used to determine LC50's. Chi-square tests ($\propto = 0.05$) were used to determine statistical significance of mortality data. Analysis of covariance and the Newman-Kuels (SNK) test was used to test differences in growth of fry in Kepone at $\alpha = 0.01$.

Results

Adult Exposure

Kepone was toxic to and was accumulated by adult sheepshead minnows exposed for four weeks (Table 1). Mortality increased in relation to the increase in concentration and duration of exposure (Figs. 1 & 2). All fish exposed to 7.8 and 24 μg of Kepone/liter of saltwater died by day 15. Twenty-two percent of fish in 0.8 μg/liter and 80% of the fish in 1.9 μg/liter died; most of the surviving fish exhibited symptoms of Kepone poisoning.

Symptoms of poisoning were related to concentration and duration of exposure, but were not typical of poisoning by other organochlorine pesticides (Henderson et al. 1959). Symptoms of poisoning progressed from scoliosis, darkening of the posterior one-third of the body, hemorrhaging near the brain and at the anterior point of darkening, to increased hemorrhaging posteriorly, swelling of the darkened area, fin rot, un-

TABLE 1. Toxicity and uptake of Kepone by adult sheepshead minnows (*Cyprinodon variegatus*) exposed for 28 days in an intermittent flow toxicity test. A total of 108 fish were exposed to 0.05 µg/liter Kepone and 32 males and 32 females were exposed to other concentrations. Samples from 1.9 µg/liter consisted of eight females and their eggs and five males; other samples consisted of a minimum of 16 females and their eggs and ten male fish.

Exposure Concentration (µg/liter)			Concentration in Fish (µg/g, Wet Weight)		
Desired	Measured	Mortality %	Females	Males	Eggs
Control	ND*	5	ND*	ND	ND
0.1	0.05	5	0.35	0.25	0.26
0.32	0.16	0	0.90	0.65	1.0
1.0	0.80	22	3.6	2.5	4.7
3.2	1.9	80	12.	11.	11.
10.0	7.8	100	–	–	–
32.0	24.	100	–	–	–

* ND = Kepone not detected: <0.02 µg/liter, <0.02 µg/g.

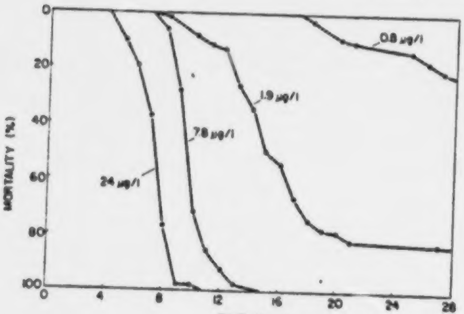

FIG. 1. Mortality of adult sheepshead minnows (*Cyprinodon variegatus*) exposed continuously to Kepone for 28 days.

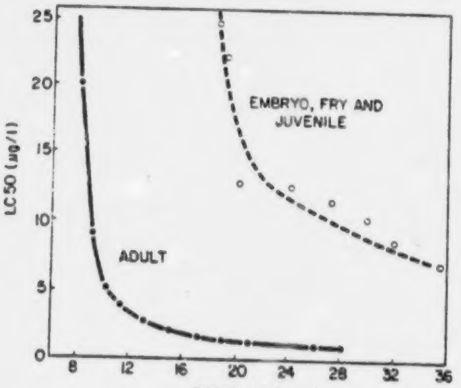

Fig. 2. Concentration of Kepone in water lethal to 50% (LC50) of embryo, fry, and juvenile sheepshead minnows continuously exposed for 36 days and adults exposed for 28 days.

coordinated swimming, and cessation of feeding. These symptoms were observed on the first day in 24 µg/liter, the second in 7.8 µg/liter, the third in 1.9 µg/liter, and the eleventh day in 0.8 µg Kepone/liter of water. These symptoms increased in severity and frequency before death from five to eight days later (Fig. 1).

Chemical analyses of adult sheepshead minnows exposed to Kepone for four weeks showed that Kepone was bioconcentrated in proportion to the concentration in the exposure water (Table 1). Concentration factors, concentration in tissue divided by concentration in water, averaged 5,200 (range 3,100 to 7,000). Concentrations of Kepone in females were greater than in males. Concentrations in eggs were generally similar to amounts in females. The concentration of Kepone in dead fish was similar to that found in fish surviving the exposure (Table 1). Dead fish in 7.8 µg of Kepone/liter of water contained 17 µg/g; in 1.9 µg/liter, 10 µg/g; in 0.8 µg/liter, 3.4 µg/g.

EMBRYO, FRY, AND JUVENILE EXPOSURE

Mortality. The effect of Kepone in water on mortality of embryos, fry, and juvenile sheepshead minnows, tested by exposing progeny of adults from the control aquarium to zero, 0.08, 0.18, 0.72, 2.0, 6.6, and 33 µg of Kepone/liter of water, was less than that observed with adult fish exposed to similar concentrations (Table 2, Fig. 2). The 36-day LC50 to juveniles exposed to Kepone in water was 6.7 µg/liter (95% confidence interval: 4.7-13.4 µg/liter) and the 28-day LC50 to adults was 1.3 µg/liter (95% confidence interval: 1.1 to 1.5 µg/liter). Kepone in water affected mortality of embryos prior to hatching. Chi-square tests revealed that mortality of embryos, in each Kepone concentration did not differ from that of controls; however, comparisons between embryo mortality in control, 0.08, and 0.18 µg/liter (average 9%) with concentrations greater than 0.18 µg/liter (average 17%) indicate that embryo mortality was significantly increased by Kepone. Fry from the embryos exposed to 6.6 and 33 µg/liter were visibly affected within 24 hours of hatching. Juvenile fish exposed to 2.0 or 0.72 µg/liter beginning as embryos did not appear visibly affected until 10 and 16 days after hatching, respectively. Symptoms of poisoning in fry less than one week old included diminished activity, loss of equilibrium, cessation of feeding, and emaciation. Symptoms in fry older than one week were identical to those observed in adult

TABLE 2. Mortality of embryo, fry, and juvenile sheepshead minnows and bioconcentration of Kepone in 19 to 63 juvenile fish (average 50) in a 36-day exposure. In some instances parental fish were exposed to Kepone for 28 days thus their eggs contained Kepone.

Exposure Concentration (μg/liter, measured)		Mortality (%)			Concentration in Juveniles (μg/g, Wet Weight)
Embryo, Fry, & Juveniles	Parental Fish	Embryos	Fry and Juvenile Fish	Total	
Control (ND)[a]	Control (ND)	6.2	3.8	10.0	ND[a]
Control (ND)[a]	0.05	8.8	3.8	12.6	ND
Control (ND)[a]	0.16	5.0	12.5	17.5	ND
Control (ND)[a]	0.80	11.5	0	11.5	ND
Control (ND)[a]	1.9	25.0	3.8	28.8	0.13
0.08	Control (ND)	11.1	11.1	22.2	1.1
0.08	0.05	8.8	0	8.8	1.6
0.18	Control (ND)	8.8	3.8	12.5	1.4
0.18	0.16	6.2	11.2	17.5	1.0
0.72	Control (ND)	21.5	6.3	27.8	2.6
0.72	0.80	13.9	3.8	17.7	1.9
2.0	Control (ND)	15.8	24.4	40.2	7.8
2.0	1.9	26.2	36.2	62.5	8.4
6.6	Control (ND)	17.5	22.5	40.0	22.
33.	Control (ND)	13.8	86.2	100	–

[a] ND = not detectable, <0.02 μg/l, <0.02 μg/g.

fish except for lack of hemorrhaging and the presence of edema in fry exposed to 0.72, 2.0 or 6.6 μg/liter.

Exposure of adult sheepshead minnows to Kepone affected mortality of their embryos in Kepone-free water (Table 2). Sixteen percent of the embryos spawned by adult fish exposed to 1.9 μg/liter failed to develop normally and died; embryos from adults exposed to lesser concentrations developed normally. Fertilization, cleavage, gastrulation, and early differentiation of the embryo including formation of somites, eyes, and circulatory system, appeared normal for about 48 hours. Thereafter, development ceased and although some embryos survived for nine days, all eventually died. Teratogenicity of Kepone might be related to its effect on the gametes or to its presence within the embryo.

In some instances, the presence of Kepone in eggs and water affected fry and juveniles to a greater extent than in water or eggs alone; effect on embryos was not increased (Table 2). Fourteen percent of the embryos spawned by adults exposed to 1.9 μg/liter developed abnormally and died. Mortality of embryos to hatching averaged 7.1% in 0.18 μg/liter or less of Kepone and 20.1% in concentrations greater than 0.18 μg/liter. Fry from adults exposed to 1.9 μg/liter showed symptoms of poisoning one day after hatching in 2.0 μg/liter and as juveniles began to die 10 days later; symptoms were more pronounced and deaths occurred ten days earlier than those for juveniles of unexposed parents. The combined effect of Kepone in eggs and water was negligible at lower concentrations.

Although juveniles that survived 36 days of exposure to 0.08 μg/liter of Kepone showed no symptoms of Kepone poisoning, three of five juvenile fish recovered at termination of the test had scoliosis and blackened tails. These five fish, spawned naturally by adult fish previously exposed to 0.05 μg/liter, were exposed to Kepone longer than were juveniles from the 36-day duration embryo, fry, and juvenile exposure. In Kepone-free water scoliosis persisted for more than 10 days. We believe, therefore, that long-term effects of Kepone on juvenile fish were underestimated in the 36-day test.

Growth. Kepone affected growth of sheepshead minnows (Fig. 3). The average standard length of juveniles exposed to 0.08 to 6.6 μg of Kepone/liter of water was less than that of unexposed juveniles ($P < 0.000000$). Juvenile length decreased in direct proportion to increased concentration of Kepone and was generally uninfluenced by a history of previous exposure of their parents. Lengths of juveniles in Kepone-free water did not differ even when parents were exposed to Kepone concentrations of 0.05 to 0.8 μg/liter. However, juveniles from parents exposed to 1.9 μg/l were shorter than unexposed juveniles ($p < 0.01$).

Bioconcentration. Chemical analyses of juvenile sheepshead minnows exposed to Kepone in water as embryos and fry for 36 days showed that Kepone was bioconcentrated and that prior exposure of parental fish had little effect on quantities concentrated (Table 2). Concentrations of Kepone in juveniles increased with increased concentrations of Kepone in water. Concentration factors increased from 3,600 to 20,000 (average 7,200) as exposure concentration decreased (Fig. 4). This relationship was not observed with adult sheepshead minnows exposed to Kepone. Juvenile progeny of adult fish exposed to 1.9 μg of Kepone/liter—though hatched and grown in Kepone-free water—contained 0.13 μg Kepone/g or about 46% of the 0.011 micrograms of Kepone originally in an egg remained in the juveniles even after 36 days in Kepone-free saltwater.

Discussion

The hazard of Kepone to fish is greatly underestimated by acute toxicity tests and is incompletely revealed by the results of our tests. Although the 96-hour LC50 of Kepone to sheepshead minnows is 70 μg/liter (Schimmel and Wilson 1977), adult fish in this study

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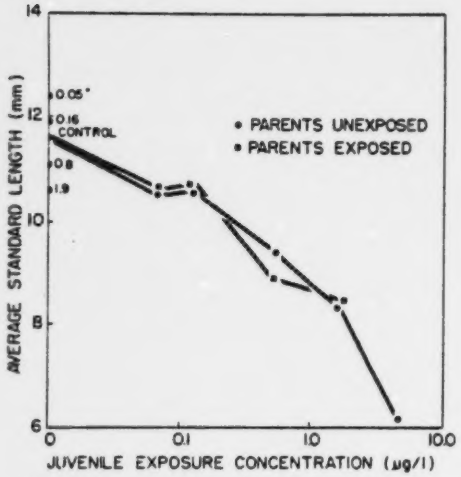

Fig. 3. Average standard length of juvenile sheepshead minnows exposed as embryos and fry for 36 days to zero, 0.08, 0.18, 0.72, 2.0, or 6.6 µg of Kepone/liter of water. Concentrations to which parent fish were exposed were: zero, 0.05, 0.16, 0.80, or 1.9 µg/liter. * Concentration of Kepone in water, µg/liter, for parent fish exposed prior to placement of their embryos in Kepone-free water.

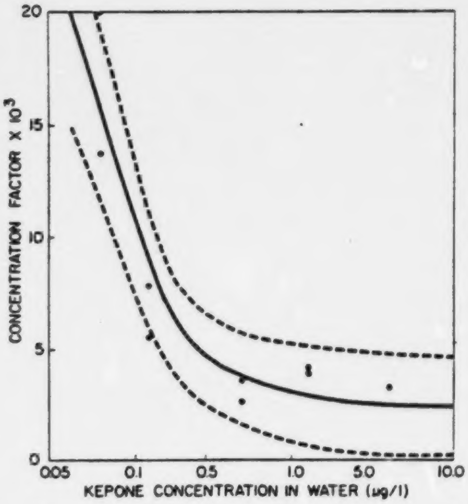

Fig. 4. Concentration factor of Kepone in juvenile sheepshead minnows after 36 days exposure that began with embryos as a function of concentration of exposure. (Concentration factor = concentration measured in juvenile fish divided by concentration measured in water). Dashed lines indicate 95% confidence limits.

died when exposed to concentrations as low as 0.8 µg/liter for 28 days. In exposures that began with embryos, juvenile fish exposed to 0.08 µg/liter were smaller than unexposed control fish. Other effects, such as scoliosis were also observed in these fish. Growth, reproduction, or survival was affected by all concentrations tested. It is generally accepted that 0.01 of the acute toxicity of a persistent organic chemical to an organism should be protective of a species (NAS-NAE 1973). Our data, however, indicate that a Kepone concentration of 0.001 of the 96-hour LC50 for sheepshead minnows affected this species detrimentally in chronic toxicity test.

Reproduction of adult sheepshead minnows was affected by exposure to Kepone in water, but the effect appears to be less pronounced than in birds and mammals fed this insecticide. Some sheepshead minnow embryos from exposed parents developed abnormally, although the majority developed, hatched, and survived as successfully as embryos from unexposed parents. Effects on quail, pheasants, and pigeons have ranged from complete inhibition of reproduction to effects which include cellular, physiological, and endocrinological alterations (De Witt et al. 1961; Elder 1964; McFarland and Lacy 1969; and Eroschenko and Wilson 1975). Kepone in food fed to cows was bioaccumulated with no apparent ill effect (Smith and Arant 1967), whereas reproduction in mice was reduced as ingested concentrations increased (Good et al. 1965). Our use of a hormone to enhance egg production and artificial spawning procedures may have masked possible additional reproductive effects of Kepone on sheepshead minnows.

Kepone is a highly bioconcentrated pesticide. Bioconcentration factors from 3,600 to 20,000 observed in juvenile sheepshead minnows exposed to Kepone were similar to those observed in juveniles similarly exposed to the insecticides endrin, 3,300–4,800 (Schimmel et al. 1975); chlordane, 8,500–12,300 (Parrish et al. 1976); toxaphene, 6,100–14,400 and heptachlor, 2,400–4,600 (Goodman et al. In press) and to the polychlorinated biphenyls, Aroclor 1016, 2,500–8,000 (Hansen et al. 1975) and Aroclor 1254, 16,000–32,000 (Schimmel et al. 1974). However, the concentration factor for Kepone in juveniles increased with decreased concentrations in water. We would expect that concentration factors would have been unaffected by changing concentrations of exposure as observed with juvenile sheepshead minnows exposed to the four insecticides and both PCB's. Concentration factors in adult sheepshead minnows exposed to Kepone, 3,100–7,000; Aroclor 1016, 4,700–14,000 (Hansen et al. 1975); and Aroclor 1254, 15,000–30,000 (Hansen et al. 1974) also were unaffected by change in concentration of exposure.

In these laboratory tests, Kepone was toxic to and accumulated by adult sheepshead minnows and their progeny. This data may be helpful in evaluating the impact of Kepone in estuaries.

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EFFECTS OF KEPONE® ON ESTUARINE ORGANISMS[1]

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ABSTRACT

Laboratory toxicity tests were conducted to determine the effects and accumulations of Kepone in estuarine algae, mollusks, crustaceans, and fishes. Nominal Kepone concentrations calculated to decrease algal growth by 50% in static bioassays lasting seven days were: 350 µg/ℓ, *Chlorococcum* sp.; 580 µg/ℓ, *Dunaliella tertiolecta*; 600 µg/ℓ, *Nitzschia* sp.; and 600 µg/ℓ, *Thalassiosira pseudonana*. Measured Kepone concentrations calculated to cause 50% mortality in flowing-seawater toxicity tests lasting 96 hours were: 10 µg/ℓ for the mysid shrimp (*Mysidopsis bahia*); 120 µg/ℓ for the grass shrimp (*Palaemonetes pugio*); >210 µg/ℓ for the blue crab (*Callinectes sapidus*); 70 µg/ℓ for the sheepshead minnow (*Cyprinodon variegatus*); and 6.6 µg/ℓ for the spot (*Leiostomus xanthurus*). Bioconcentration factors (concentration in whole animals divided by concentration measured in water) in these tests were greatest for fishes (950 to 1,900) and less for grass shrimp (420 to 930).

Survival, growth, and reproduction of mysids and sheepshead minnows were decreased in chronic bioassays lasting 14 to 64 days. Growth of mysids and sheepshead minnows was reduced by exposure to 0.07 µg/ℓ and 0.08 µg/ℓ respectively. Bioconcentration factors for sheepshead minnows in the chronic bioassay averaged 5,200

®Registered trademark, Allied Chemical Corp., 40 Rector St., New York, 10006. Kepone was purchased from Chem Service, West Chester, PA, as 99% pure. Our analyses indicated 88% purity.

[1] Contribution No. 311, Environmental Research Laboratory, Gulf Breeze.

(range, 3,100-7,000) for adults exposed for 28 days and 7,200 (3,600-20,000) for juveniles exposed for 36 days. The chronic toxicity and bioconcentration potential of Kepone are more important factors than its acute toxicity in laboratory evaluations of environmental hazard. Therefore, these factors should be considered when attempting to assess present impacts and to limit future impacts of this insecticide on the aquatic environment.

INTRODUCTION

Kepone (decachlorooctahydro-1,3,4-metheno-2H-cylobuta [cd] pentalene 2-one) is an insecticide that was manufactured and formulated in the United States to control ants, cockroaches, and insect pests of potatoes and bananas. Kepone is toxic to birds and mammals, including man (Jaeger 1976), and acutely toxic to some estuarine organisms (Butler 1963). Recent contamination of water, sediment, and biota in freshwater and estuarine portions of the James River, Virginia, has stimulated concern about this chemical's hazard to aquatic biota (Hansen et al. 1976). This concern was based on (1) the continued occurrence of Kepone in many finfishes and shellfishes in amounts that forced closure of fishing because of potential human health hazard, and (2) laboratory studies which showed that Kepone is highly bioaccumulative and toxic to estuarine organisms, particularly in chronic exposures. This paper describes the results of these laboratory toxicity tests with estuarine algae, oysters, crustaceans, and fishes and chronic tests with a crustacean and a fish.

EXPERIMENTAL PROCEDURES

Acute Toxicity

Algae: The unicellular algae *Chlorococcum* sp., *Dunaliella tertiolecta*, *Nitzschia* sp., and *Thalassiosira pseudonana* were exposed to Kepone for seven days to determine its effect on growth (Walsh et al. 1977). Algae were cultured in 25 or 50 ml of growth media and artificial seawater of 30 ^{0}/oo salinity and a temperature of 20 C (Hollister et al. 1975). Kepone, in 0.1 ml acetone, was added to culture media, and 0.1 ml of acetone was added to control cultures. Photoperiod consisted of 12 hours dark and 12 hours of 5000 lux illumination. Effect on growth was determined by electrophotometrically measuring optical density. Also, algae grown for 6 days in media and then exposed to 100 μg/ℓ Kepone for 24 hours were analyzed for Kepone content.

Oysters: The acute toxicity of Kepone to embryos of the eastern oyster (*Crassostrea virginica*) was determined by measuring its effect on development

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of fully-shelled, straight-hinged veligers in a 48-hour static exposure[1]. Methods used were those of Woelke (1972) and U. S. EPA (1975). Test containers were 1-ℓ glass jars that contained 900 mℓ of 20 C, 20 ⁰/oo salinity seawater and 25,000 ± 1,000 oyster embryos. All test concentrations were triplicated. The number of normal and abnormal embryos were counted microscopically in a Sedgewick-Rafter cell at the end of 48 hours of exposure to Kepone.

Crustaceans and Fishes: The acute toxicity of Kepone to grass shrimp (*Palaemonetes pugio*), blue crabs (*Callinectes sapidus*), sheepshead minnows (*Cyprinodon variegatus*), and spot (*Leiostomus xanthurus*) was determined in 96-hour flow-through toxicity tests (Schimmel and Wilson 1977). Acclimation and testing procedures were compatible with those of Standard Methods (APHA 1971). Test animals were caught locally and 20 were placed in each 18ℓ aquarium. Water flow to each aquarium was 68 ℓ/hour. Stock solutions of Kepone in acetone were metered into experimental aquaria at the rate of 60 mℓ/hour. Control aquaria received 60 mℓ of acetone/hour. At the end of the experiment, surviving animals were chemically analyzed for Kepone content.

The acute toxicity of Kepone to mysids (*Mysidopsis bahia*) was determined by using intermittent flows of water from a diluter (Mount and Brungs 1967) or continuous flow of water from a siphon and Kepone from an infusion pump (Bahner et al. 1975). Thirty-two 48-hour-old juvenile mysids were placed in chambers (4 mysids per chamber) in each test aquarium. Chambers consisted of glass petri dishes to which a 15 cm tall cylinder of 210μ mesh nylon screen was glued. Water in the chambers was renewed by a self-starting siphon which nearly emptied and then filled each aquarium at about 25 min intervals.

Chronic Toxicity

Mysidopsis bahia: The chronic toxicity of Kepone to this mysid was determined in 19-day exposures that began with 48-hour-old juveniles. (Nimmo et al., in press). The time permitted production of several broods for assessment of reproductive success and survival of progeny. Exposure conditions, apparatus, and number of mysids per concentration were identical to those of the acute toxicity tests. Three tests were conducted: One to [illegible], and two at lower concentrations to determine effects on growth. Data from the two growth experiments were pooled for statistical analysis.

Cyprinodon variegatus: The chronic toxicity of Kepone to sheepshead minnows was determined in a 64-day flow-through bioassay--exposure of adults for 28 days followed by a 36-day exposure of their progeny (Hansen et al. 1977). We delivered Kepone, 0.0088 μℓ of the solvent triethylene glycol, and 1.5ℓ of filtered 30 C seawater (average salinity, 15 ⁰/oo; range, 8-26 ⁰/oo) to each 70ℓ aquarium during each of 440 daily cycles of the dosing apparatus of Schimmel et al. (1974). Seawater and solvent were delivered to the control aquarium. Thirty-two adult females and 32 adult males were

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[1] This research was performed under an EPA contract by Tom Heitmuller, Bionomics-EG&G, Inc. Marine Research Laboratory, Pensacola, Florida 32507.

exposed to each concentration of Kepone for 28 days. Egg production was enhanced using injections of 50 I.U. of human chorionic gonadotrophic hormone on exposure day 25 and 27 (Schimmel et al. 1974). Eggs were fertilized on day 28 and placed in chambers (glass petri dishes with 9-cm tall cylinders of 450μ nylon mesh). Twenty embryos were used in each chamber. Embryos from control fish were placed in four chambers in the control aquaria and in four chambers in each of the six aquaria receiving Kepone. Embryos from fish in each of the six aquaria receiving Kepone were placed in four chambers in that aquarium and in four chambers in the control aquarium. Water in the chambers was exchanged by the action of a self-starting siphon in each aquarium that caused water levels to fluctuate 5 cm about 40 times per day. In the 36-day exposure to determine Kepone's effect on survival and growth of progeny, embryos hatched and fry grew until they were juvenile fish. Kepone content of adult fish, their eggs, and juvenile fish was determined.

STATISTICAL ANALYSES

Probit analyses of growth and mortality data were used to determine EC50's and LC50's. Growth data for *M. bahia* were subjected to analysis of variance ($\alpha = 0.05$) and for *C. variegatus*, analysis of covariance and Newman-Kuels tests ($\alpha = 0.01$) was used.

CHEMICAL ANALYSES

Water from acute and chronic tests with crustaceans and fishes, and organisms surviving these tests, were analyzed by gas chromatography. Methods of extraction concentration, cleanup, and quantification were described by Schimmel and Wilson (1977).

RESULTS AND DISCUSSION

Acute Toxicity

Algae: Growth of marine unicellular algae was reduced by exposure to Kepone in static tests (Table 1). *Chlorococcum* was the most sensitive of the four algae tested with a 7-day EC50 of 350 μg/ℓ. The three less sensitive species responded similarly to Kepone with overlapping confidence limits for EC50's. Algae exposed to 100 μg Kepone/ℓ of media accumulated the chemical with *Chlorococcum* containing 0.80 μg/g; *D. tertiolecta*, 0.23 μg/g; *Nitzschia*, 0.41 μg/g; and *T. pseudonana*, 0.52 μg/g. Butler (1963) reported that when estuarine phytoplankton were exposed to 1,000 μg/ℓ carbon fixation was reduced by 95%.

Oysters: The 48-hr EC50 for oyster larvae in static tests was less than those of algae (Table 1). The EC50, calculated using nominal water concentrations, was 66 μg/ℓ[2]. Embryos from 56 μg/ℓ were fully shelled and straight-hinged but appeared smaller than those from controls. The percentage of normal embryos in 65 μg/ℓ was 32 percent and in 87 μg/ℓ it was 0%. The concentration of Kepone calculated to reduce shell deposition of juvenile eastern oysters by 50% in a 96-hour flowing water bioassay was 38 μg/ℓ in water of 14 C and 11 μg/ℓ in water of 31 C (Butler 1963).

TABLE 1. ACUTE TOXICITY OF KEPONE TO ESTUARINE ORGANISMS. ALGAL AND MOLLUSK TOXICITY TESTS WERE STATIC AND ESTIMATED NOMINAL CONCENTRATIONS REDUCING GROWTH OF ALGAE AND EMBRYONIC DEVELOPMENT OF OYSTERS BY 50% (EC50). TOXICITY TESTS WITH CRUSTACEANS AND FISHES WERE FLOW-THROUGHS THAT ESTIMATED THE MEASURED CONCENTRATION IN WATER LETHAL TO 50% (LC50). NINETY-FIVE % CONFIDENCE LIMITS ARE IN PARENTHESES.

Organisms	Temperature, C	Salinity, $^{o}/oo$	Exposure Duration, Days	EC50/LC50 µg/ℓ	
Algae					
Chlorococcum sp.	20	30	7	350	(270-400)
Dunaliella tertiolecta	20	30	7	580	(510-640)
Nitzschia sp.	20	30	7	600	(530-660)
Thalassiosira pseudonana	20	30	7	600	(500-700)
Mollusk					
Crassostrea virginica	20	21	2	66	(60-74)
Crustaceans					
Callinectes sapidus	19	20	4	>210	
Mysidopsis bahia	26	13	4	10	(8.1-12)
Palaemonetes pugio	20	16	4	120	(100-170)
Fishes					
Cyprinodon variegatus	18	15	4	70	(56-99)
Leiostomus xanthurus	25	18	4	6.6	(5.3-8.8)

Crustaceans and Fishes: Kepone, at the concentrations tested, was acutely toxic to mysids (Nimmo et al. 1977), grass shrimp, sheepshead minnows, and spot, but not to blue crabs (Schimmel and Wilson 1977) (Table 1). Spot and mysids were the more sensitive species with 96-hour LC50 values of 6.6 and 10 µg/ℓ. Crabs exposed to as much as 210 µg Kepone/ℓ suffered no significant mortality. Symptoms of acute Kepone poisoning in fishes included lethargy, loss of equilibrium, and darkened coloration on the posterior portion of the body, occasionally only in one quadrant. Crustaceans became lethargic before death but exhibited no color change. Butler (1963) reported 48-hour LC50 or EC50 values (based on nominal concentrations) for other estuarine organisms were: brown shrimp (*Penaeus aztecus*), 85 µg/ℓ; and white mullet (*Mugil curema*), 55 µg/ℓ.

Kepone was bioconcentrated from water by all four species we exposed for 96 hours. Bioconcentration factors (concentration in tissue divided by measured Kepone in water) for fishes were similar (950 to 1,900). Bioconcentration factors for grass shrimp ranged from 420 to 930 and for blue crabs, 6 to 10.

CHRONIC TOXICITY

Mysidopsis bahia: Exposure of this mysid to Kepone for 19 days in the first experiment decreased its survival and reduced the number of young produced per female (Table 2) (Nimmo et al. 1977). At the highest concentration (8.7 μg/ℓ) all mysids were dead within the first two days. At lesser concentrations (1.6 and 4.4 μg/ℓ) mortality continued throughout the test. Eighty-four % of the mysids survived exposure to 0.39 μg Kepone/ℓ water and 91% survived in control aquaria. In addition, natural reproduction was affected. Average number of young mysids produced per female was 15 in control, 9 in 0.39 μg/ℓ, and 0 in 1.6 μg/ℓ. Mysids that survived throughout the Kepone exposure appeared smaller than those in control aquaria, therefore, two additional experiments were conducted to measure Kepone's effect on growth.

TABLE 2. EFFECT OF KEPONE ON THE SURVIVAL OF *MYSIDOPSIS BAHIA* AND ON AVERAGE NUMBER OF YOUNG PER FEMALE IN A 19-DAY FLOW-THROUGH TOXICITY TEST.

Average Measured Kepone Concentration (μg/ℓ)	Percentage Survival	Number of Young per Female
Control	91	15.3
0.39	84	8.9*
1.6	50	0
4.4	3	--
8.7	0	--

*Statistically significant at α = 0.05 using 2 sample t-test.

In these experiments, the average length (tip of carapace to end of uropod) of mysids exposed to Kepone was decreased (Nimmo et al. 1977). Females exposed to 0.072, 0.11, 0.23, or 0.41 μg/ℓ were significantly shorter than were control mysids; average length was 8.2 mm for exposed versus 8.6 mm for control female mysids. Unexposed and exposed males, however, were of similar average lengths, 7.7 to 8.0 mm.

Cyprinodon variegatus: Kepone was toxic to adult sheepshead minnows exposed for 28 days (Table 3). Symptoms of poisoning included: scoliosis, darkening of the body posterior to the dorsal fin, hemorrhaging near the brain, edema, fin-rot, uncoordinated swimming, and cessation of feeding. Symptoms were first observed on day 1 in 24 μg/ℓ, 2 in 7.8 μg/ℓ, 3 in 1.9 μg/ℓ, and day 11 in 0.8 μg/ℓ. Mortalities began 5 to 8 days after onset of symptoms.

TABLE 3. EFFECT OF KEPONE ON AND ACCUMULATION OF KEPONE BY ADULT SHEEPSHEAD MINNOWS EXPOSED FOR 28 DAYS.

Average Measured Exposure Concentration, µg/ℓ	Percentage Mortality	Whole Body Concentration, µg/g
ND*	5	ND
0.05	5	0.30
0.16	0	0.78
0.80	22	3.0
1.9	80	12.
7.8	100	
24.	100	

*ND = Kepone not detected in control water (<0.02 µg/ℓ) nor in control fish (<0.02 µg/g).

Kepone affected the progeny of 28 day exposed adults. In Kepone-free water, mortality of embryos from adults exposed to 0.05-0.8 µg/ℓ was similar to that of embryos from unexposed adults (range, 6-12 percent). However, in Kepone-free seawater, 25% of the embryos from fish exposed to 1.9 µg of Kepone/ℓ died; abnormal development of 13 of these 20 embryos preceded mortality.

Kepone in water affected progeny of exposed parents to a greater extent than progeny of unexposed parents (Table 4). Some embryos exposed to 2.0 µg/ℓ developed abnormally and fry had more pronounced symptoms and they began to die 10 days earlier when parental fish had been exposed to 1.9 µg/ℓ than was observed in progeny from unexposed parents.

Kepone also affected growth of sheepshead minnows in the 36-day exposure of progeny (Figure 1). The average standard length of juveniles exposed to all Kepone concentrations was less than that of unexposed control juveniles. Lengths decreased in direct proportion to increasing Kepone concentrations in water and were generally not influenced by parental exposure. A similar decrease was also noted in weights, but because juveniles exposed to 0.72, 2.0, or 6.6 µg/ℓ were edematous, they weighed more than unexposed juveniles of similar lengths.

Kepone was bioconcentrated by sheepshead minnow adults and their progeny exposed to the insecticide in water. Kepone was bioconcentrated in adult fish in direct proportion to concentration in exposure water (Table 3). Concentration factors averaged 5,200 (range, 3,100-7,000). Kepone concentrations in females and their eggs were similar and were 1.3 times greater than amounts in males. Concentrations of Kepone in juvenile fish, at the end of the 36-day progeny exposure, increased with increased concentration of Kepone in water (Table 4). Prior exposure of parental fish apparently did not affect final Kepone concentration in progeny. Concentration factors for juvenile fish averaged 7,200 (range, 3,600-20,000) and increased with decrease in concentration of exposure.

TABLE 4. MORTALITY IN PROGENY OF ADULT SHEEPSHEAD MINNOWS THAT WERE EXPOSED TO KEPONE AND IN PROGENY OF UNEXPOSED, CONTROL FISH. NOMINAL EXPOSURE FOR THE 28-DAY EXPOSURE OF ADULT FISH AND THE 36-DAY EXPOSURE OF PROGENY WERE THE SAME. PROGENY EXPOSURE BEGAN WITH EMBRYOS AND ENDED WITH JUVENILE FISH FROM THE EMBRYOS. RESIDUES ARE CONCENTRATIONS OF KEPONE (μg/g) IN WHOLE JUVENILES, WET WEIGHT.

Measured Exposure Concentration	Parental Fish History			
	Progeny of Unexposed Parents		Progeny of Exposed Parents	
μg/ℓ	Mortality %	Residue μg/g	Mortality %	Residue μg/g
Control (ND)	10	ND[1]	10	ND[1]
0.08	22	1.1	9	1.6
0.18	12	1.4	18	1.0
0.72	28	2.6	18	1.9
2.0	40	7.8	62	8.4
6.6	40	22.	--	--
33.	100	--	--	--

[1] ND = not detectable, <0.02 μg/ℓ, <0.02 μg/g.

In our tests, Kepone was acutely toxic to, and accumulated by, estuarine algae, mollusks, crustaceans, and fishes. Chronic toxicity tests with *M. bahia* and *C. variegatus* revealed that Kepone affected survival, growth, and reproduction. Effects on growth were observed at 0.001 of the 96-hour LC50. Accumulation of Kepone was also greatest in chronic tests. Therefore, chronic tests should be used to assess Kepone's environmental hazard and to make decisions necessary to minimize its future impact on the aquatic environment.

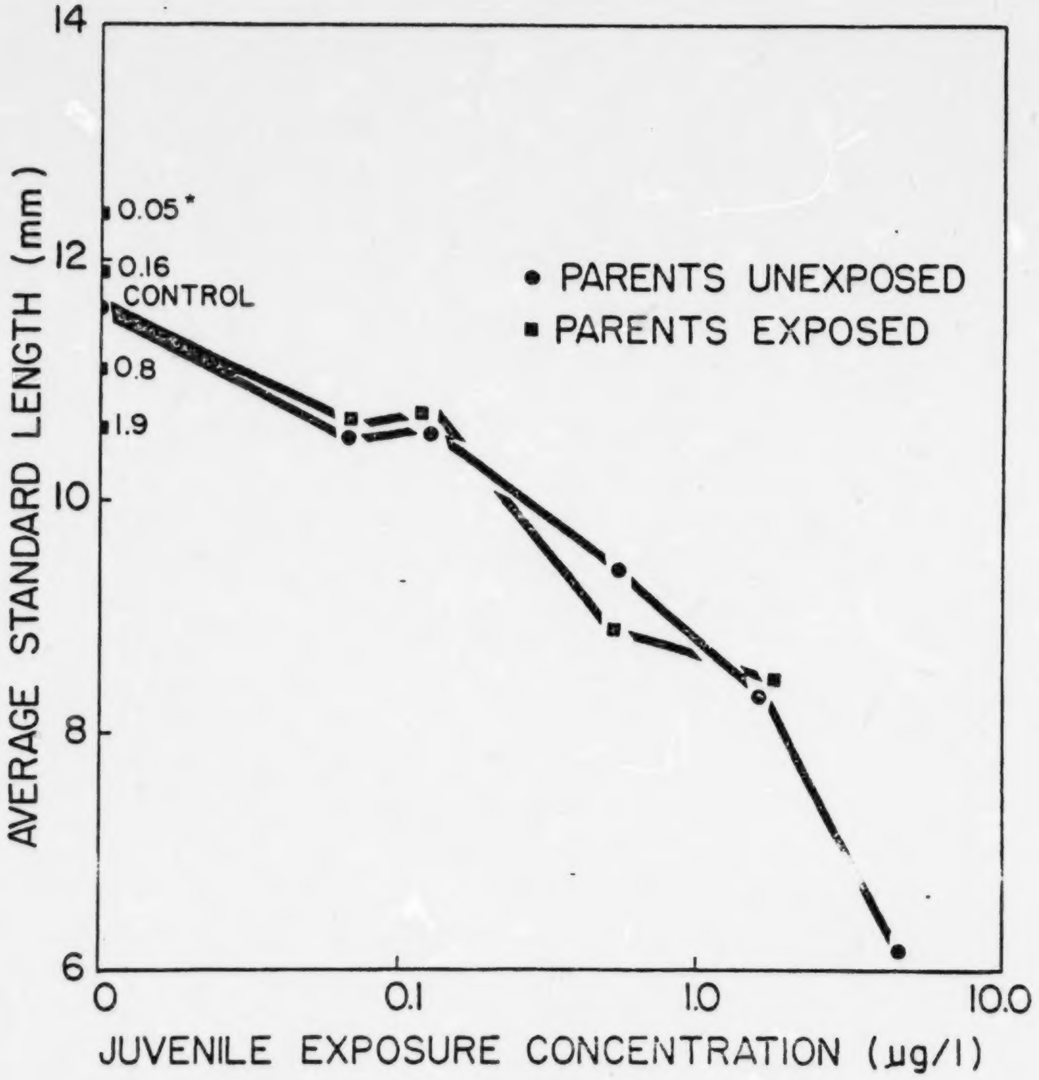

Figure 1. Average standard length of juvenile sheepshead minnows exposed as embryos, fry, and juveniles for 36 days to 0, 0.08, 0.18, 0.72, 2.0, or 6.6 µg of Kepone/ℓ of water. Parent fish in some instances also were exposed to similar concentrations of Kepone: 0, 0.05, 0.16, 0.80, or 1.9 µg/ℓ.

*Concentration of Kepone in water, µg/ℓ, for parent fish exposed prior to placement of their embryos in Kepone-free water.

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SCIENCE

Kepone-Induced Scoliosis and Its Histological Consequences in Fish

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Kepone-Induced Scoliosis and Its Histological Consequences in Fish

Abstract. *Scoliosis in fish is caused by several diverse agents that possibly act on the central nervous system, neuromuscular junctions, or ionic metabolism. The organochlorine pesticide Kepone induces scoliosis in the sheepshead minnow. Some effects associated with Kepone-induced scoliosis in these fish are disruption of myotomal patterns, inter- and intramuscular hemorrhage, fractured centra of vertebrae, and death. The histological syndrome of Kepone poisoning in fish and the clinical syndrome in humans suggest that the nervous system is a primary target for Kepone and that scoliosis is a secondary effect of Kepone poisoning in fish.*

We have found that exposure of sheepshead minnows (*Cyprinodon variegatus*) to a relatively low concentration of the organochlorine Kepone (decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*c,d*]pentalen-2-one) produces a syndrome in the fish in which scoliosis, resulting in severe spinal column injury, is one cardinal sign. Scoliosis, lateral curvature of the spine, has been reported to occur in several species of fish as a result of dietary deficiencies (*1*), organophosphate and carbamate poisoning (*2*), heavy metal exposure (*3*), and parasitic infections (*4*). This report is concerned with the severe histological effects of scoliosis in sheepshead minnows exposed to Kepone in the laboratory.

Hansen and co-workers (*5*) first observed scoliosis and blacktail (loss of neurologic control of melanocytes in the caudal region) as a syndrome of Kepone exposure in sheepshead minnows. They experimentally demonstrated that induction of scoliosis with Kepone was a dose-dependent, time-related phenomenon.

Scoliosis occurred in sheepshead minnows exposed 1 day to 24 μg of Kepone per liter, 2 days to 7.8 μg of Kepone per liter, 3 days to 1.9 μg of Kepone per liter, and 11 days to 0.8 μg of Kepone per liter. All tests were laboratory-controlled flowing seawater exposures.

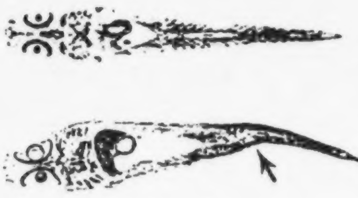
We repeated exposures of sheepshead minnows to Kepone in the laboratory to obtain specimens exhibiting scoliosis for histological study. Twenty juvenile and ten adult sheepshead minnows were exposed for 17 days to flowing seawater containing 4 μg of Kepone per liter at 21° to 30°C and 23.5 to 29 parts per thousand salinity. Triethylene glycol was the solvent carrier for Kepone. A second equivalent group of control fish received only the solvent carrier chemical in flowing seawater.

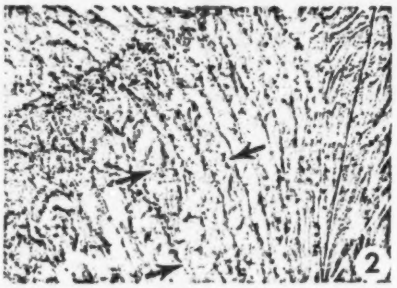
All exposed fish exhibited signs of Kepone poisoning by day 10 of exposure. These fish demonstrated scoliosis, blacktail, loss of equilibrium, sporadic hyperkinesis, and tetanic convulsions. Living specimens with various degrees of scoliosis were selected from day 10 to day 17 of exposure for standard histological fixation and processing. Fish were fixed in Davidson's fixative, embedded in paraffin, and serially sectioned at 7 μm—some through a longitudinal, horizontal plane (Fig. 1, a and b) and others through a sagittal-parasagittal plane. Normal control fish were processed similarly. Selected fish with scoliosis and control fish were xeroradiographed for study of spinal column form.

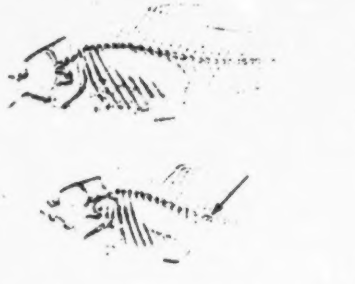
Histological study revealed that fish with incipient scoliosis had precaudal trunk flexures resulting from long-term rigorous contractions of skeletal muscle in myotomes on one of either side of their bodies (Figs. 1a and 2). Xeroradiographs show that the major spinal column flexure occurs in the vicinity of, and involves, vertebrae 17 through 21 (cephalic to caudal count). We have counted 26 to 27 nonfused vertebrae in several normal specimens of the sheepshead minnow (Fig. 3). Normal muscle bundle patterns are broken and myotome boundaries (septa) are obscured by the abnormally contorted muscle bundles (Fig. 2).

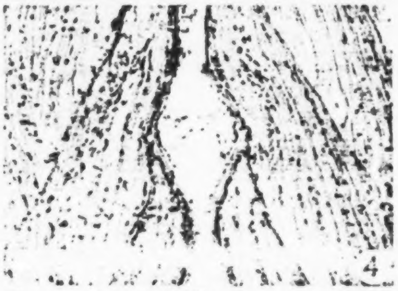
In more advanced cases of scoliosis, hemorrhagic foci often occurred in affected myotomes, suggesting a rupture of minor vessels or capillaries possibly caused by severe contortion of muscle. The most striking effect observed, always in severely scoliotic fish, was the breaking of the centra of vertebrae at the epicenter of flexure in the spinal column (Figs. 4 and 5). This was accompanied by apparent osteoblastic repair tissue (Fig. 5, arrows) suggesting that the fractures occurred sometime prior to fixation of the fish for histology. Fish with fractured vertebrae also had the greatest displacement and disorientation of myotomal musculature (Fig. 5). Obstruction (pinching) of the dorsal aorta or caudal artery and severe imposition of displaced bone into the neural canal were further concomitant results of severe scoliosis (Fig. 6). Fish thus affected were paralyzed and probably would have died.

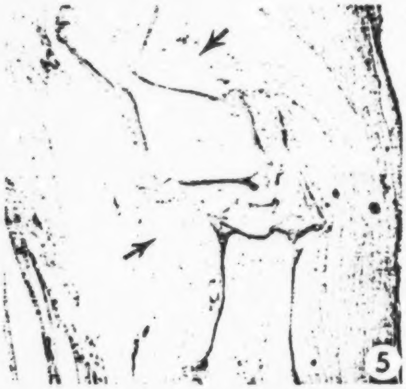
No histological lesions in the central nervous system that could have initiated tetany or paralysis were observed. We propose, however, that scoliosis is secondary to tetany and paralysis of trunk musculature initiated by an undetermined molecular neurological or neuromuscular dysfunction (or both), probably directly caused by Kepone. The appearance of blacktail (loss of neurologic control of caudal melanocyte patterns) preceding, during, and after scoliosis strengthens the possibility that the initiating lesion is neurologic (6). Another possibility is that systemic calcium metabolism may have been affected by Kepone. Evidence from living and fixed fish











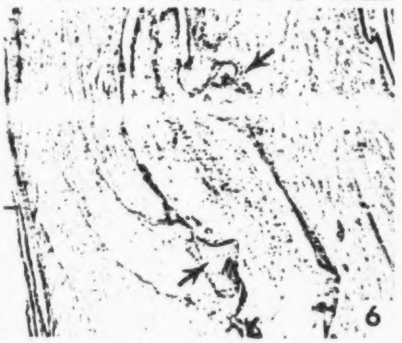


Fig. 1. (a) Longitudinal histological section from the horizontal plane of normal control fish; section is at level of spinal column (×3.5). (b) Longitudinal horizontal plane section of fish with advanced scoliosis; the concave region in trunk (arrow) is location of flexure (×3.5). Fig. 2. Contorted muscle bundles (arrows) in myotome in concave region shown in Fig. 1b; the contorted musculature reflects the tetanic paroxysms that lead to scoliotic flexure of the trunk. Compare Figs. 2 and 4 (×160). Fig. 3. Xeroradiographs of normal sheepshead minnow (a) and sheepshead minnow with incipient scoliosis (b); the region of early spinal column flexure (arrows) shows obvious loss of vertebral periodicity. Fig. 4. Horizontal plane section from normal fish showing normal alignment of vertebral centra and normal myotomes in the region of trunk affected by Kepone-induced scoliosis in exposed fish (×160). Fig. 5. Fractured centra of vertebra in severely scoliotic fish exposed to 4 μg of Kepone per liter for 17 days; distortion of myotomes and foci of osteoblastic repair tissue at points of breaks in centrum walls is shown (arrows) (×64). Fig. 6. Horizontal section through the spinal cord of fish used in Fig. 5 with fractured vertebra; intrusion of disoriented bone (arrows) from neural arch of vertebra against lateral funiculi of spinal cord is shown (×64).

indicate that trunk muscles are unable to regain normal tonus and form (Fig. 2). Tetanic convulsions or chronic muscular rigor (or both) are associated with scoliosis and probably produce the fractured vertebrae of severely affected fish. The possible effects that Kepone may have on fish calcium metabolism, on the corpuscles of Stannius (because of their calcium-mediating role in some fishes), and on muscle contraction have not yet been evaluated.

The mechanism or mechanisms whereby different organochlorine compounds affect organisms are poorly understood. Human victims of Kepone poisoning have suffered tremors, nervousness (hyperkinesis), loss of memory, and slurred speech, among other effects (*7*). The human response syndrome suggests neurological lesions, some of which probably occur at higher nervous centers, as a result of Kepone poisoning. Tremors and other neurological-dependent responses in laboratory animals increased in severity with increasing Kepone concentration and duration of exposure (*7*). Hansen, *et al.* (*5*) observed the same correlation between concentration of Kepone, duration of exposure, and severity of scoliosis and related signs in fish. Our observations suggest that the severity of scoliotic effects in the sheepshead minnow is related to the duration of continuous exposure to a single low Kepone concentration (4 μg/liter). Much higher concentrations of Kepone (2 to 400 mg/kg per day) are required to elicit neuropathological, reproductive, and tissue effects in birds or mammals (*7*).

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Kepone® Bioconcentration, Accumulation, Loss, and Transfer through Estuarine Food Chains.[1]

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ABSTRACT: Accumulation, transfer, and loss of Kepone in estuarine organisms were studied in laboratory bioassays. Kepone was bioconcentrated by oysters (*Crassostrea virginica*), mysids (*Mysidopsis bahia*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*), and spot (*Leiostomus xanthurus*), from concentrations as low as 0.023 μg/l seawater. Bioconcentration factors ranged from 10 to 340 in static exposures and 900 to 13,500 in flow-through bioassays, and were dependent on species and exposure duration.

Depuration of Kepone from oysters in Kepone-free water was rapid (35% loss in 24 hours); however, depuration of Kepone was slow in crustaceans and fish, with tissue concentrations decreasing 30–50% in 24–28 days.

Oysters, fed *Chlorococcum* containing approximately 34 μg Kepone/g wet weight, attained 0.21 μg Kepone/g (wet tissue) in 14 days, but when fed Kepone-free plankton, depurated Kepone to below detectable concentrations (<.02 μg/g) within 10 days.

Spot obtained Kepone when fed live mysids that had grazed on Kepone-laden brine shrimp. Kepone residues (1.05 μg/g wet tissue) in these fish approached the concentration of their food (1.23 μg/g wet tissue); at the lower concentration tested. Kepone concentrations below detection limits (<.2 μg/g) in prey accumulated in the predator to detectable concentrations (0.02 μg/g) within 30 days. Bioaccumulation factors (concentration of Kepone in predator/concentration in prey) at 30 days were equal (0.85 spot/mysid; 0.53 mysid/brine shrimp) in the high and low concentrations tested. The initial bioconcentration of Kepone from water by plankton was the dominant source of Kepone to each member of this food chain, but significant (>85%) quantities of Kepone transferred from prey to predatory fish.

Introduction

Contamination of the James River water, sediments, and biota with Kepone has prompted research to help define the routes of transfer of the insecticide from water through selected estuarine trophic levels. Biota of the James River estuary and Chesapeake Bay contain Kepone (Hansen et al. 1976), apparently due to transport of the chemical downstream from the freshwater portion of the river. Since no convenient method existed to assess the rate of Kepone movement in the biota of the James River and Chesapeake Bay, laboratory bioconcentration from water and bioaccumulation from food experiments were designed to determine the rates and magnitudes of Kepone accumulated from water and food by selected estuarine organisms. It is important to determine the accumulation of Kepone from water and food by various estuarine species, so that the information can be used in the decision-making processes that may affect the water quality for the biota in the Chesapeake Bay region, or limit transfer of Kepone to seafoods consumed by man. The alga, oyster, mysid, shrimp, and fish used in

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[1] Contribution No. 294, Environmental Research Laboratory, Gulf Breeze, FL.

our experiments are representative of many ecologically important species. The top consumers in our food chains tested are endemic to both the Chesapeake Bay area and northern Gulf of Mexico and are commercially important human food items. *Leiostomus xanthurus*, the top carnivorous species of one laboratory food-chain, provides an estimated 21.5 million pounds annual recreational catch for fishermen of the Middle Atlantic states (U.S. Department of Commerce 1975).

This study provides information about: (1) the rates and magnitudes of Kepone accumulation from water by estuarine biota; (2) rates of Kepone depuration by animals in Kepone-free water; and (3) rates of Kepone transfer through laboratory food chains.

Methods

BIOCONCENTRATION OF KEPONE FROM WATER BY ESTUARINE ORGANISMS

Oysters

Eastern oysters (*Crassostrea virginica*) were exposed to Kepone (88% pure) in a 56-day, flow-through bioassay to determine the rates of uptake and depuration of this insecticide. Seawater (mean temperature 14.2 C, range 9–20 C; mean salinity 15 ‰, range 4–22 ‰) was pumped from Santa Rosa Sound, Florida, into a constant-head trough in the laboratory. Approximately 440 l/hour was delivered by siphons to each of three 166 l aquaria. Oysters (100/aquarium) were not fed but could obtain plankton from the unfiltered seawater in which they were held. Stock solutions of Kepone in triethylene glycol (TEG) were metered into the two experimental aquaria at the rate of 10 ml/day. Measured concentrations of Kepone in the two experimental aquaria were 0.39 and 0.03 μg/l seawater. A control aquarium received 10 ml TEG/day.

Oysters (56 mm to 92 mm, umbo to distal valve edge height; $\bar{x}$ = 71.8 mm) were collected, acclimated to laboratory conditions for ten days, exposed to Kepone for 28 days, and then held for 28 days in Kepone-free seawater. Five oysters were sampled from each aquarium at 4 hours, 8 hours, 1 day, 8 days, and twice weekly thereafter to day 28. During the 28-day depuration portion of the test, oysters were sampled at similar intervals. Analysis methods for Kepone in water and tissues (whole-body, wet weight) were those of Schimmel and Wilson (1977).

Crustaceans

Mysids (*Mysidopsis bahia*), collected from laboratory cultures (Nimmo et al. 1977), were exposed to average measured concentrations of 0.026 or 0.41 μg Kepone/l seawater for 21 days (mean temperature 27.2 C, range 26–29 C; mean salinity 18 ‰, range 12–26 ‰) and in a second experiment, grass shrimp (*Palaemonetes pugio*), seined and acclimated to experimental conditions for 10 days, were exposed to average measured concentrations of 0.023 or 0.40 μg Kepone/l seawater in a 28-day, flow-through bioassay (mean temperature 27 C, range 26–29 C; mean salinity 25 ‰, range 21–28 ‰). Experimental methods used were those of Bahner et al. (1975). The grass shrimp were held for an additional 28-day period in clean seawater to assess depuration of the insecticide. Filtered seawater at a rate of approximately 60 l/hour flowed through each aquarium containing mysids or grass shrimp. Mysids and shrimp were fed 48-hour-old *Artemia* nauplii daily. Kepone content of mysids and shrimp was determined weekly during exposure and depuration. In a third experiment, grass shrimp were collected by seine from the Lafayette River estuary near Norfolk, Virginia, and were held in flowing seawater (mean temperature 25.5 C; mean salinity 15 ‰) in the laboratory to determine the extent of depuration of Kepone from field-exposed shrimp. These shrimp were analyzed for Kepone concentrations on days 7, 11, 17, and 21 after being transferred to flowing Kepone-free water in our laboratory.

Fishes

Sheepshead minnow (*Cyprinodon variegatus*) adults, acclimated to laboratory test conditions, were exposed to an average measured concentration of 0.05 μg Kepone/l of water (mean temperature 30 C;

mean salinity 15 ‰, range 8–26 ‰) for 28 days, using the methods of Hansen et al. (1977) and were held in Kepone-free water for an additional week. Ten fish, generally five females and five males, were sampled on days 0, 1, 3, 7, 14, 21, and 28 of exposure to Kepone and on day 7 of depuration.

Spot (*Leiostomus xanthurus*) were seined, acclimated for 10 days, exposed to average measured concentrations of 0.029 or 0.4 μg Kepone/l of filtered flowing seawater (mean temperature 23 C, range 21–24 C; mean salinity 18 ‰, range 9–24 ‰) for 30 days and allowed to depurate the chemical for 24 days. Composite samples of three fish were sampled each week for residue analysis. Spot, exposed to 0.4 μg Kepone/l seawater and allowed to depurate for 24 days, were dissected into liver, brain, gills, muscle, and offal (rest of body tissues) and analyzed for Kepone.

Fillets (including scaleless skin) and the remaining portions of sheepshead minnows and spot (wet weight) were analyzed for Kepone content. The data were summed to calculate concentrations in whole fish.

BIOACCUMULATION OF KEPONE IN FOOD CHAINS CONSISTING OF ESTUARINE ORGANISMS

Algae-Oyster Food Chain

The green alga, *Chlorococcum* sp., contaminated with Kepone was used as food for oysters to determine if contaminated phytoplankton could be a significant source of the pesticide to oysters. *Chlorococcum* sp. was grown for 6 days in one liter of culture medium in 2800-ml Fernbach flasks according to the method of Hollister et al. (1975). Random cultures were dosed with 0.1 mg Kepone in acetone, while others served as controls after treatment with acetone alone. After 24 hours of exposure, algal cultures (mean wet weight = 0.15 g) were harvested by centrifugation and washed three times by resuspension in clean growth medium and centrifugation. The cells were resuspended in 5 l of seawater and fed to oysters by using the methods of Bahner and Nimmo (1976). Samples of the algae were analyzed daily for Kepone.

Rate of Kepone accumulation was determined by allowing oysters to feed on control or Kepone contaminated green algae in flowing seawater. Oysters for this study were collected and acclimated for 10 days to laboratory conditions in flowing seawater. Twenty-four oysters were placed in each of two aquaria (one control and one experimental) that received 60 l filtered seawater/hour (mean temperature 22 C, range 21–23 C; mean salinity 19 ‰, range 15–24 ‰) and were fed approximately 50 ml of the appropriate (control or contaminated) algal suspension at 15-minute intervals for 14 days. A 10-day depuration period followed the feeding study during which the oysters received raw, unfiltered, flowing seawater and no additional *Chlorococcum*. Oysters (n = 3 per sample) were analyzed for Kepone content on days 0, 7, 10, 14, 17, and 24 of the experiment.

Plankton-Mysid-Fish Food Chain

Transfer of Kepone from water to plankton to mysids to fish was investigated by feeding living brine shrimp nauplii that were contaminated with 2.33 μg Kepone/g tissue to mysids, that were then fed to spot, the top predator of this laboratory food chain. Juvenile spot were seined and acclimated according to the methods previously described for sheepshead minnows and spot. Mysids (*Mysidopsis bahia*), the intermediate food organism, were collected from laboratory cultures. Commercially available brine shrimp eggs were hatched during 48 hours in clean seawater or in seawater to which 0.005 or 0.1 mg Kepone/l was added (Bahner and Nimmo 1976). The brine shrimp were harvested daily and served as the "planktonic" food for the mysids. Approximately 40 mysids were distributed among each of six compartments of two 30-l aquaria. The compartments were separated by coarse nylon screen that allowed for flow of water and *Artemia* throughout each aquarium while confining the mysids to separate compartments. Mysids that had fed on *Artemia* for 72 hours were harvested from one compartment of each holding aquarium, rinsed with seawater, and fed to the spot. Each compartment was refilled with mysids to provide for subsequent feeding periods. By this method, each of 12 juvenile spot, (average length 40 mm), in each of three 30-l glass aquaria, were fed 3 to 5 control or contaminated mysids daily. Aquaria containing spot received 60 l of seawater/hour to prevent anoxia and to minimize bioconcentration of Kepone depurated from the mysids. Water averaged 19 C (range 16–21) and 18 ‰ salinity (range 13–23 ‰). Brine shrimp, 30 to 45 mysids, 2 to 3 spot, and water from each aquarium were analyzed weekly for Kepone.

Results and Discussion

Bioconcentration from Seawater

Kepone was bioconcentrated from water by oysters, mysids, grass shrimp, sheepshead minnows, and spot in all concentrations tested (Figs. 1, 2, 3, 4, and 5; Table 1), and all species showed nearly equilibrated tissue concentrations of Kepone within 8 to 17 days after exposure to Kepone began in water. Bioconcentration factors for Kepone in these species ranged from 2,300 to 13,500 in long-term (>96 hrs) flow-through bioassays (Table 2). Kepone bioconcentrated in oysters to approximately 10,000 times the concentration in the exposure water within 19 days. Mysids bioconcentrated Kepone up to 13,000 times the amount measured in the exposure water. Each mysid (mean live-weight = 2.5 mg for 66 adults), exposed to 0.026 μg Kepone/l for 14 days contained approximately 5.9 ng Kepone; therefore, this amount of the chemical could enter food chains of estuarine predators that consumed each mysid. Stomachs of flounders from Chesapeake Bay (standard length 25 to 174 mm) con-

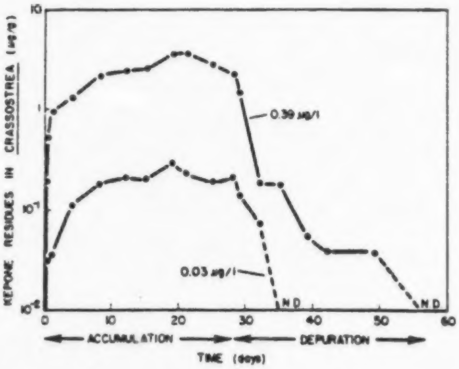

Fig. 1. Bioconcentration of Kepone from water containing average measured concentrations of 0.03 or 0.39 μg/l by oysters (*Crassostrea virginica*) exposed for 28 days, and its depuration by oysters placed in Kepone-free water for 28 days (mean temperature 14.2°C; mean salinity 15 ‰). ND = not detectable, <0.02 μg/g wet weight.

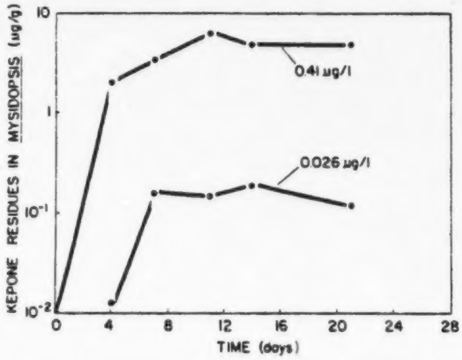

Fig. 2. Bioconcentration of Kepone from water containing average measured concentrations of 0.026 or 0.41 μg/l by mysids (*Mysidopsis bahia*) exposed for 21 days (mean temperature 27.2°C; mean salinity 18 ‰).

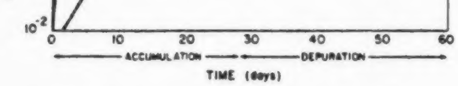

Fig. 3. Bioconcentration and depuration of Kepone in grass shrimp (*Palaemonetes pugio*) during 56-day study. Circles indicate concentrations of Kepone accumulated from water containing average measured concentrations of 0.023 or 0.4 μg/l by grass shrimp exposed in the laboratory for 28 days, and its depuration by shrimp placed in Kepone-free water for 28 days (mean temperature 27°C; mean salinity 25 ‰). Triangles indicate concentrations in shrimp collected from Lafayette River, Norfolk, Virginia and held in clean flowing seawater at the ERL, Gulf Breeze, for 21 days (mean temperature 25.5°C; mean salinity 15 ‰). Dashed line represents extrapolation to initial concentration at beginning of depuration.

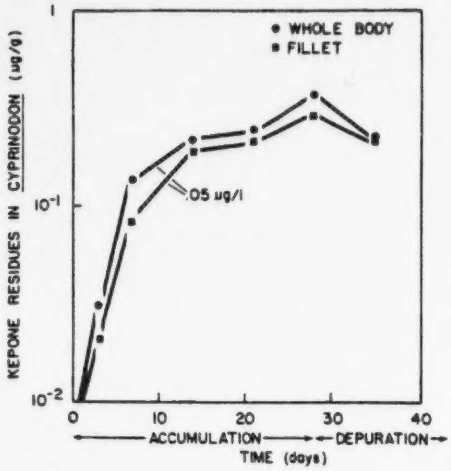

Fig. 4. Bioconcentration of Kepone from water containing average measured concentration of 0.05 µg/l by sheepshead minnows (*Cyprinodon variegatus*) exposed for 28 days, and its depuration from fish placed in Kepone-free water for 7 days (mean temperature 30°C; mean salinity 15 ‰).

tained an average of twenty mysids (Stickney et al. 1974); mysids comprised up to 14% of the diets of striped bass from the York and Rappahannock rivers. Mysids were conspicuously absent in gut analyses of James River striped bass, but decapod crustaceans (i.e. *Palaemonetes* sp.) accounted for 48% (by volume) of their diets (Markel and Grant 1970). *Palaemonetes* have one of the highest bioconcentration factors of Kepone (Table 2), and like other decapod crustaceans, are one of the species least sensitive to acute exposures of Kepone (Schimmel and Wilson 1977). Grass shrimp bioconcentrated Kepone up to 11,000 times the concentration in the exposure water. After 28 days of exposure to 0.023 µg Kepone/l, each shrimp contained approximately 8.6 ng Kepone, an amount that could be transferred to predators. Bioconcentration of Kepone was more efficient with increased concentrations in water for all crustaceans tested (Table 2).

Kepone was bioconcentrated from water by sheepshead minnows—important omnivores that link energy transfer from detritus and benthic plants and animals to carnivores in higher trophic levels. Each fish (mean weight 1.5 g) contained approximately 0.54 µg Kepone after 28 days of exposure to 0.05 µg Kepone/l seawater. Kepone concentrations were slightly higher in female sheepshead minnows (0.35 µg/g) than male fish (0.25 µg/g).

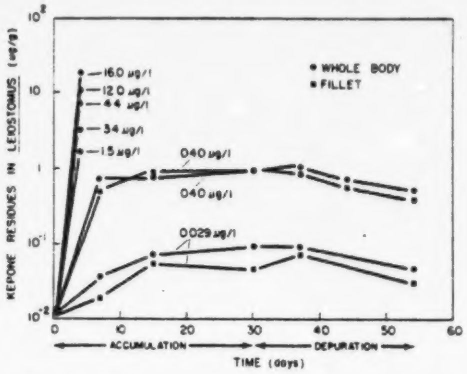

Fig. 5. Bioconcentration of Kepone from water containing average measured concentrations of 0.029, 0.40, 1.5*, 3.4*, 4.4*, 12.0*, and 16.0* µg/l by spot (*Leiostomus xanthurus*) exposed for 4 or 30 days, and its depuration by fish placed in Kepone-free water for 24 days (mean temperature 23°C; mean salinity 18 ‰). *Data from Schimmel and Wilson (1977).

Spot, a commercially valuable food fish, bioconcentrated Kepone from 0.029 µg/l seawater; each fish (mean weight 1.4 g) contained approximately 0.13 µg Kepone. The bioconcentration factors for Kepone in fish were similar to those of other chlorinated hydrocarbon insecticides (Schimmel et al. 1975; Schimmel et al. 1976). Kepone accumulated in edible fillets to near the whole-body concentrations in fish (Figs. 4, 5,& 6); therefore, one of the largest reserves (22 %) of Kepone in absolute weight is in the edible portion of contaminated fish. Although the greatest body burdens of Kepone on a wet-weight basis are in the brain, liver, and gill tissues, the relatively large size of muscle and offal tissues contributes large Kepone reserves to higher trophic levels (Fig. 6).

Depuration of Kepone was not consistent among the species tested. Clearance of the chemical from oysters was relatively rapid, with no Kepone detected within 7 to 20 days after exposure ceased (Fig. 1). Depuration of Kepone from laboratory exposed grass shrimp (Fig. 3) and fish (Fig. 4 & 5) was slower; Kepone concentrations were reduced 30–50% in 24 to 28 days. Grass

TABLE 1. Concentrations of Kepone (μg/g wet tissue) measured in oysters (*C. virginica*) exposed to 0.03 or 0.39 μg/l for 28 days, mysids (*M. bahia*) exposed to 0.026 or 0.41 μg/l for 21 days, grass shrimp (*P. pugio*) exposed to 0.023 or 0.40 μg/l for 28 days, sheepshead minnows (*C. variegatus*) exposed to 0.05 μg/l for 28 days, and spot (*L. xanthurus*) exposed to 0.029 or 0.4 μg/l for 30 days in flowing water experiments. Animals were allowed to depurate Kepone for up to 28 days in Kepone-free flowing seawater.

Duration of Exposure (Days)	Oysters 0.03 μg/l	Oysters 0.39 μg/l	Mysids 0.026 μg/l	Mysids 0.41 μg/l	Shrimp 0.023 μg/l	Shrimp 0.40 μg/l	Duration of Exposure (Days)	Minnows 0.05 μg/l	Minnows 0.05 μg/l	Spot 0.029 μg/l	Spot 0.029 μg/l	Spot 0.40 μg/l	Spot 0.40 μg/l
	Whole Body	Whole Body	Whole Body	Whole Body	Whole Body	Whole Body		Muscle	Whole Body	Muscle	Whole Body	Muscle	Whole Body
1/6	0.012	0.19					1/6						
1/3	0.031	0.53					1/3						
1	0.036	0.96	–	–	–	–	1	<0.01	<0.01				
2	–	–	–	–	<0.02	0.27	2						
3	–	–	–	–	–	–	3	0.019	0.031				
4	0.11	1.3	<0.04	2.0			4						
7	–	–	0.16	3.3	0.038	0.47	7	0.086	0.14	<0.02	0.037	0.50	0.73
8	0.18	2.2					8						
9	–	–	–	–	0.072	1.79	9						
11	–	–	0.15	6.3			11						
12	0.21	2.4					12						
14	–	–	0.19	4.7	0.088	2.18	14	0.19	0.22				
15	0.20	2.5	–	–	–	–	15	–	–	0.055	0.072	0.86	0.76
19	0.29	3.5					19						
21	0.23	3.6	0.12*	4.8*	0.12	3.15	21	0.21	0.24				
25	0.19	2.7					25						
28	0.21*	2.2*	–	–	0.087*	4.57*	28	0.29*	0.37*				
30	–	–	–	–	–	–	30	–	–	0.048*	0.093*	0.99*	0.94*
Duration of Depuration							Duration of Depuration						
1/6	0.30	2.1					1/6						
1/3	0.30	2.7					1/3						
1	0.14	1.4					1						
2	–	–	–	–	0.1	2.62	2						
4	0.074	0.18					4						
7	<0.01	0.18	–	–	0.1	2.5	7	0.21	0.22	0.074	0.093	0.87	1.04
11	<0.01	0.055					11						
14	<0.01	0.039	–	–	0.084	1.78	14	–	–	–	–	0.57	0.72
21	<0.01	0.039	–	–	0.055	1.5	21						
24	–	–	–	–	–	–	24	–	–	0.031	0.0469	0.38	0.52
28	<0.01	<0.01	–	–	0.035	0.84	28						

* Final day of exposure.

TABLE 2. Bioconcentration factors for selected species exposed to measured concentrations of Kepone in water.

Species	Exposure Concentration (μg/l)		Duration of Exposure (days)	Mean Bioconcentration Factor
Chlorococcum sp.	100.	(static)	1	340
Chlorococcum sp.	40.	(injection)	2	6,000
Crassostrea virginica	0.03		19	9,354
Crassostrea virginica	0.39		21	9,278
Artemia salina	5.	(static)	2	10
Artemia salina	100.	(static)	2	23
Mysidopsis bahia	0.026		21	5,962
Mysidopsis bahia	0.41		21	13,473
Palaemonetes pugio	0.023		28	5,127
Palaemonetes pugio	0.4		28	11,425
Cyprinodon variegatus	0.05		28	7,115
Leiostomus xanthurus	0.029		30	3,217
Leiostomus xanthurus	0.4		30	2,340
Leiostomus xanthurus	1.5*		4	1,120
Leiostomus xanthurus	3.4*		4	941
Leiostomus xanthurus	4.4*		4	1,591
Leiostomus xanthurus	12.0*		4	900
Leiostomus xanthurus	16.0*		4	1,050

* Data from Schimmel and Wilson (1977).

shrimp from the Lafayette River depurated Kepone at rates similar to those of laboratory exposed shrimp with approximately 20% of the Kepone lost during 21 days in seawater containing no Kepone. Spot that were exposed to Kepone for 30 days and allowed to depurate for 24 days, contained highest Kepone concentrations in brain, followed by liver, gills, and muscle (Fig. 6). Many chlorinated chemicals are highly concentrated in liver and other fatty tissues (Parrish et al. 1974; Parrish et al. 1975), but the unusual distribution of Kepone might be explained by its water solubility relative to other insecticides.

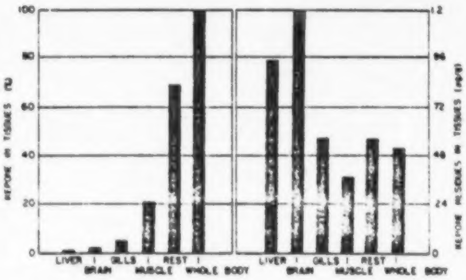

Fig. 6. Distribution of Kepone in selected tissues of spot (*Leiostomus xanthurus*). Spot were exposed to 0.4 μg Kepone/l for 30 days and allowed to depurate and equilibrate for an additional 24 days prior to sampling (mean temperature 23°C; mean salinity 18 ‰). Percentage of whole-body Kepone burden in five tissues is on left. Measured residues (wet weight) are on right.

BIOACCUMULATION OF KEPONE IN FOOD CHAINS CONSISTING OF ESTUARINE ORGANISMS

Algae-Oyster Food Chain

Chlorococcum, grown in media enriched to 0.1 mg Kepone/l, bioconcentrated the chemical to a mean of 34 μg/g in whole cells; the bioconcentration factor in these static exposures was 340X (Table 2). In preliminary tests, continuous infusion of 2.1 μg Kepone/hour into static cultures of *Chlorococcum* produced cells with bioconcentration factors near 6000 × within 48 hours; adsorption of Kepone by the cells and vessels limited the average measured concentration in the media to approximately 0.04 mg/l. Continuous exposure in this test is probably more representative of those occurring in an estuary, and places the importance of bioconcentration of Kepone by phytoplankton in proper perspective.

Oysters bioaccumulated Kepone to 0.21 μg/g when fed *Chlorococcum* sp. containing an average of 34 μg Kepone/g for 14 days (Fig. 7; Table 3). Kepone in feces and pseudofeces from these oysters averaged 1.78 μg Kepone/g (dry weight). Kepone was not detectable in the unfiltered water samples; shell growth was evident; and all oysters gained weight during the test. Weight gain

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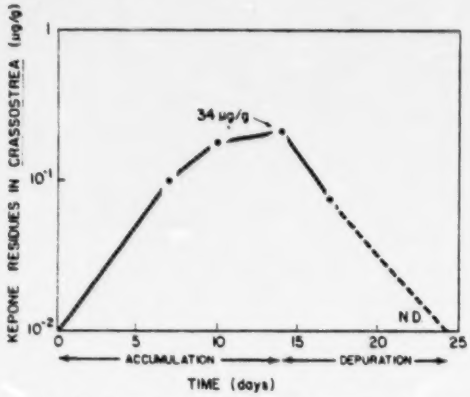

Fig. 7. Bioaccumulation of Kepone by oysters (*Crassostrea virginica*) that consumed algae (*Chlorococcum* sp.) with residues of 34 µg Kepone/g (wet weight). Oysters fed on contaminated algae for 14 days (mean water temperature 22°C; mean salinity 19 ‰), and were then fed uncontaminated plankton for an additional 10 days to allow depuration of the chemical. ND = not detectable, <0.02 µg/g wet weight.

TABLE 3. Concentrations of Kepone (µg/g whole body, wet tissue) measured in oysters (*C. virginica*) fed algae (*Chlorococcum* sp.) containing 34 µg/g for 14 days and in spot (*L. xanthurus*) fed mysids (*M. bahia*) containing 0.02 µg/g (estimated) or 1.03 µg/g for 30 days in flowing water experiments. Oysters were fed uncontaminated plankton for an additional 10 days to allow depuration of the chemical.

Duration of Exposure (Days)	Oysters fed 34 µg/g	Spot fed 0.02 µg/g (Estimated)	Spot fed 1.03 µg/g
	µg/g Whole Body	µg/g Whole Body	µg/g Whole Body
0	<0.02	<0.02	<0.02
5	–	<0.02	0.095
7	0.10	–	–
10	0.18	<0.02	0.15
14	0.21*	–	–
20	–	<0.02	0.59
30	–	0.015,0.024*	1.0,1.1*
Duration of Depuration			
3	0.075	–	–
10	<0.02	–	–

* Final day of exposure.

TABLE 4. Kepone transfer in algae-oyster food chain. Algae (*Chlorococcum* sp.) grown in Kepone enriched media for 24 hrs was fed to oysters (*C. virginica*) for 14 days in flow-through feeding experiment.

	Control food chain	Exposed food chain
(1) Kepone (single dose) in algal media (mg/l)	Control	0.1
(2) Kepone residues in algae after 24 hrs of exposure (mg/kg)	Control (ND)*	$\bar{X} = 34$
(3) Bioconcentration factor from water [(2)/(1)]	–	340
(4) Kepone residues in oysters after 14 days of feeding	Control (ND)*	0.21
(5) Bioaccumulation factor from algae to oysters [(4)/(2)]	–	.007
(6) Food chain factor [(4)/(1)]	–	2.1

* ND = non-detectable (<0.02 mg/kg).

in exposed oysters was not different than control oysters (analysis of covariance, $\alpha = 0.05$). Kepone concentrations in oysters fed the contaminated algae appeared to almost reach equilibrium in 14 days; but quantity of Kepone transferred from these algae to oysters was limited, probably due to rapid depuration of the chemical from the oysters. Kepone was not detectable (<0.02 µg/g) 10 days after the oysters received no contaminated food. Most Kepone was depurated from oysters within 96 hours; therefore, if oysters in the natural environment contain measurable Kepone residues, a recent or continuous source of Kepone from water and/or food has been available.

The maximum overall accumulation and transfer of Kepone or food-chain factor from water to algae and finally to oysters was 2.1 (Table 4). The food-chain factor was obtained by dividing the concentration of the contaminant in the final consumer by the concentration of the contaminant in the water of the primary producer. The concentration measured in each consumer can be compared with a lower trophic level to determine the bioaccumulation factor of the contaminant for that predator-prey pair. Bioaccumulation factor is similar to bioconcentration factor, but the contaminant is in food and is consumed by the predator. The bioaccumulation factor for oysters consuming algae under these test conditions was only 0.007 (Table 4). These data indicate that transfer of Kepone from algae by oysters was inefficient, or that the uptake was masked by the oyster's ability to depurate the chemical quickly.

Plankton-Mysid-Fish Food Chain

Spot accumulated Kepone by consuming live mysids that had grazed on Kepone-laden brine shrimp (Fig. 8; Tables 3, 5). Brine shrimp exposed to 0.1 mg Kepone/l seawater contained whole-body residues of 2.33 μg/g after 48 hours. Mysids that fed for 72 hours on these brine shrimp contained 1.23 μg Kepone/g. Kepone concentrations in spot that consumed the mysids for 30 days were slightly less than those in the mysids (Fig. 9), but uptake of Kepone exceeded depuration in fish as indicated by the positive slope of the uptake curves. The failure for residues to reach equilibrium during the test could be attributed to the slow depuration of Kepone from fish tissues.

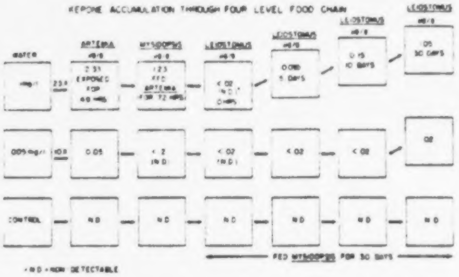

Fig. 8. Block diagram of four-level food chain. Nominal concentrations of Kepone in water (left) were control (bottom), 0.005 mg/l (center), and .1 mg/l (top). *Artemia salina* nauplii, hatched in these concentrations of Kepone, were fed to mysids (*Mysidopsis bahia*), and mysids were fed to spot (*Leiostomus xanthurus*) for 30 days. Average measured concentrations of Kepone in each trophic species is given. Mean water temperature was 19°C and mean salinity 18 ‰.

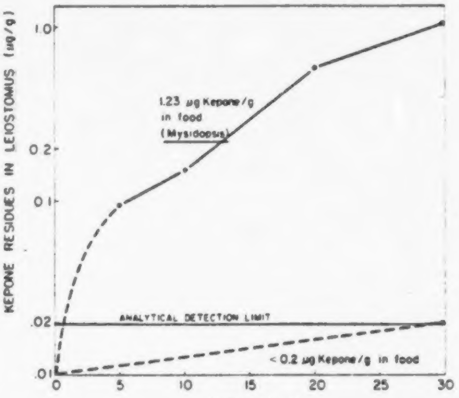

Fig. 9. Bioaccumulation of Kepone by spot (*Leiostomus xanthurus*) fed contaminated mysids (*Mysidopsis bahia*) containing average measured whole-body residues of <.2 (0.023 estimated) or 1.23 μg Kepone/g wet tissue. Detection limit for Kepone in spot tissues was ≤0.02 μg/g. Mean water temperature was 19°C and mean salinity 18 ‰.

TABLE 5. Kepone transfer in plankton-mysid-fish food chain. Brine shrimp (*A. salina*) were hatched during 48 hrs in Kepone enriched seawater and were fed to mysids (*M. bahia*) for 72 hrs. Mysids were then fed to spot (*L. xanthurus*) for 30 days in flow-through feeding experiment.

	Control food chain	Low exposure food chain	High exposure food chain
(1) Kepone (single dose in brine shrimp media (mg/l)	Control	0.005	0.1
(2) Kepone residues in brine shrimp after 48 hrs of exposure (mg/kg)	Control (ND)*	0.049 0.043 0.058 x̄ = 0.050	1.3 2.4 3.3 x̄ = 2.33
(3) Bioconcentration factor from water [(2)/(1)]	–	10.	23.3
(4) Kepone residues in mysids after 72 hrs of feeding (mg/kg)	Control (ND)*	x̄ = 0.023 (estimated)	0.89 1.0 1.8 x̄ = 1.23
(5) Bioaccumulation factor from brine shrimp to mysids [(4)/(2)]	–	0.5 (estimated)	0.53
(6) Kepone residues in spot after 30 days of feeding (mg/kg)	Control (ND)*	0.015 0.024 x̄ = 0.0195	1.0 1.1 x̄ = 1.05
(7) Bioaccumulation factor from mysids to spot [(6)/(4)]	–	>0.85 (estimated)	>0.85
(8) Food chain factor [(6)/(1)]	–	>3.9	>10.5

* ND = non-detectable (<0.02 mg/kg).

Mysids, which consumed *Artemia* with residues of 0.05 or 2.33 μg Kepone/g (wet weight), attained 0.023 (estimated) or 1.23 μg Kepone/g whole-body residues within 72 hours. The estimated 0.023 μg Kepone/g whole-body mysids, obtained by assuming the bioaccumulation factor of 0.85 (Table 5) for Kepone transfer from mysids to fish as observed in the food chain beginning with 0.1 mg/l, also occurred in the food chain that began with 0.005 mg Kepone/l. The residue in mysids was then estimated to be 0.023 μg Kepone/g, which was consistent with the estimated bioaccumulation factor of 0.5 for Kepone transfer from *Artemia* to mysids for 72 hours. The food-chain factors were different for this food chain (3.9 compared to 10.5), since the bioconcentration factor for Kepone by brine shrimp from the lower concentration in water (0.005 mg/l) was less than that from the higher concentration (0.1 mg/l). The initial bioconcentration of Kepone from water by planktonic food organisms was the dominant source of Kepone to each member of this food chain, since bioaccumulation factors were less than unity.

In the field, lower concentrations of Kepone in seawater could result in a duplication of Kepone residues found in the fish of this food chain because the plankton could be expected to be chronically exposed to the contaminant. Thus, the food-chain factor would be expected to increase in the natural environment since bioconcentration factors for a chlorinated hydrocarbon pesticide (DDT) in feral plankton have been shown to exceed 4000X (Cox 1971). However, bioconcentration of Kepone could overshadow the amount of the chemical received from food by the animals in this food chain. Approximately 3,000 times as much Kepone in food as in water was needed to produce similar concentrations in spot in 28 to 30 days. Therefore, bioconcentration of Kepone was dominant in this food chain, but significant quantities (>85%) of Kepone transferred from prey to predatory fish. Rapid uptake from water and food, slow depuration, and appreciable solubility in water indicate that Kepone will transfer through food webs and pose threats to consumers.

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Effects of Kepone on Estuarine Microorganisms

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INTRODUCTION

Aquatic microorganisms undoubtedly play a critical role in the metabolic transformation of organic and inorganic compounds which enter lakes, estuaries, and oceans. Some of these compounds, particularly the chlorinated hydrocarbon group, degrade very slowly in nature and consequently their fate and accumulation are of great importance in determining environmental quality. Since many chlorinated hydrocarbons accumulate in terrestial and aquatic ecosystems, they could eventually alter basic microbial degradation processes if the compounds were toxic to the microorganisms. For example, interferences in the competition among bacteria in a natural population, due to the elimination of an important member of that population, could reduce transformation efficiency and even effect the extent of transformation. The possibility that pesticide residues may have deleterious effects on microorganisms and their activities has received considerable attention (Alexander 1968, Edwards 1972, Tu and Miles 1976, Ware and Roan 1970). The emphasis of these studies, however, has been on the effects of pesticide residues on soil rather than aquatic microorganisms. Although the effects of pesticides on phytoplankton and photosynthesis has been documented (Ware & Roan 1970), information is lacking on the effects of organochlorines on aquatic microorganisms and the processes they mediate. Several important organochlorine compounds have been tested for their toxicity to aquatic microorganisms. Bourquin et al. (1975) and Bourquin and Cassidy (1975) have shown polychlorinated biphenyls to be toxic to a variety of bacteria isolated from estuarine waters. Using the agar-disc diffusion method, the investigators found that 0.1 mg of Aroclor 1242 per disc elicited definite zones of inhibition. They also showed that, by documenting physiological characteristics relative to toxicant-sensitive and toxicant-insensitive groups, certain characteristics correlated with one group more than another. Richards (1977) has shown that pentachlorophenol in flowing sea water systems caused the selection of PCP-tolerant microorganisms. Brown et al. (1975b) have shown that mirex was nontoxic to aquatic bacteria but Kepone, a contaminant of mirex, was toxic. Kepone may be a chemical breakdown product of mirex (Carlson et al. 1976).

Kepone[R] (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalene-2-one), an insecticide, has been shown to be an extensive contaminant of the James River System in Virginia (U.S. EPA, 1975). Its introduction into the James River was due to industrial effluents. Recent studies have shown that Kepone is toxic to algae (Walsh et al. 1977) and several estuarine animals (Schimmel and Wilson 1977). This paper reports on the toxicity of Kepone to estuarine bacteria.

MATERIALS AND METHODS

Bacterial Cultures

Bacterial isolates obtained from Range Point salt marsh (Pensacola Beach, Florida), Escambia Bay surface water, and Sabine Island disposal pond were used in disc-agar sensitivity assays. These pure cultures of bacteria and fungi were originally isolated from batch culture enrichments on various carbon sources and maintained as part of our laboratory stock culture collection. Samples of water and sediment from these waters and the Gulf of Mexico were used in all mixed-culture toxicity testing. Isolates from these environments were used to determine the effects of Kepone on growth and the oxidation of specific substrates.

[R] Registered trademark, Allied Chemical Corp., New York. Mention of trade names in this publication does not constitute endorsement by the U.S. Environmental Protection Agency.

Low concentrations of the insecticide Kepone, approaching those found in contaminated James River sediment, were shown to be inhibitory to the growth and oxygen uptake of microorganism randomly selected from estuarine environments. No significant correlations were noted between growth inhibition by Kepone and cell morphology, aliphatic hydrocarbon utilization, pesticide tolerance, selected enzyme activities, nitrate reduction, and urea hydrolysis. Oxygen uptake by pure cultures grown on glucose or hydrocarbons at cell densities equivalent to 10^3 - 10^4 cells/ml was decreased by 60-100% at Kepone concentrations of 0.02 - 2.0 mg/l. Total viable counts from estuarine water or sediments grown aerobically on agar media containing 0.02 mg/l Kepone were reduced by 8 - 78 °/oo. The inhibitory effect was partially eliminated when sediment populations were grown anaerobically.

Reagents and Chemicals

Kepone (98% pure) was obtained from Chem Services, Inc., West Chester, PA. N,N-dimethylforamide (DMF) was purchased from Aldrich Chemical Co., Milwaukee, Wisc. Microbiological media and substrates were obtained from Difco Laboratories, Detroit, MI.

Media

Zobell's 2216 marine medium Z-15 (Aaronson 1970) was prepared at a final salinity of 15 $^{o}/oo$ using Rila Marine Mix (Rila Products, Teaneck, N.J.). Solid medium was prepared with 2% Bacto-agar. Minimal salts basal medium (MSB) was prepared according to Stanier et al. (1966). Glucose (0.2%) was added to MSB as a growth substrate and 0.01% yeast extract (Difco) was added to compensate for any auxotrophic cultures.

Kepone-Marine agar medium was prepared by adding desired concentrations of Kepone (in acetone carrier) to Zobell's medium after autoclaving. Acetone control plates (no Kepone) were used in all experiments.

Pure Culture Inhibition Studies: Disc Sensitivity Assays

Pure cultures, grown in Zobell's (Z-15) broth for two 18 hr growth cycles were spread on Z-15 agar plates. Three filter paper discs (Schleicher & Schuell, 12.7 mm, No. 740-E, Keene, NH), designed for antibacterial substrate testing, were saturated with acetone or DMF solutions containing 0.01, 0.1, or 1.0 mg Kepone per milliliter, dried, and then placed on the inoculated agar plate. A fourth disc contained only DMF or acetone. The actual volume of Kepone solution per disc was calculated to be 0.11 μl by determining the average increase in weight of a DMF or acetone saturated disc.

Mixed Culture Inhibition Studies

Water and sediment samples collectd from James River, VA., and various estuarine and marine areas near Pensacola Beach were serially diluted in water blanks of the appropriate salinity and plated on Z-15 agar plates with and without Kepone. The plates were incubated aerobically at 28°C and colonies were counted at 4- and 8-day intervals. Sediment samples were similarly plated and incubated anaerobically in BBL Disposal Anaerobic Systems (BBL, Baltimore, Md.) at 28° for 8 days. Dominant colony types from the aerobic Kepone containing plates were picked with sterile toothpicks and spotted onto Z-15 agar. The resulting colonies were replicated onto various differential and selective media for physiological characterization.

Oxygen Uptake Studies

Selected pure cultures aerobic Kepone plates were grown in 100 ml of either Z-15 broth or MSB broth with glucose or succinate (0.2%) for 18 hr prior to harvesting by centrifugation (10Xg, 10 min.). The cells were washed once with 0.05 M K_2HPO_4 buffer (pH 7.5) containing 1.5% NaCl and finally resuspended in 2 ml of saline buffer. Suspensions were kept in an ice bath until used. Oxygen uptake was determined by a Gilson Oxygraph (Model K-ICT-C, Gilson Electronics, Middleton, Wisc.) equipped with a Clark electrode. The reaction vessel contained 1.7 - 1.8 ml phosphate buffer, 10 - 50 μℓ washed cell suspension (100-300 μg protein), and 10 - 50 mg oxidizable substrate. After a baseline level of oxygen uptake was established, 10 μℓ of Kepone solution (0.19 - 19.0 μg Kepone/ℓ DMF) was added to the reaction vessel and the oxygen uptake observed for any resulting effects. A positive control, 10 μℓ of a pentachlorophenol solution (20 mM), was then put in the same reaction vessel.

Methods

Physiological characterization of isolates on differential and selective media was carried out according to the methods of Colwell and Weibe (1970). The ability of isolates to grow on hydrocarbons (hexadecane, undecane, octadecane, benzene, naphthalene, biphenyl, xylene, toluene) was determined by placing hydrocarbon-saturated filter paper discs in the lids (for volitale hydrocarbons) or on the agar (for nonvolitale hydrocarbons) of MSB agar plates streaked with the appropriate isolate. The appearance of growth on the agar which was greater than the control plates was taken to be hydrocarbon utilization by the isolate since no other carbon source was supplied. Pesticide tolerance tests (using toxaphene, Arochlor 1242, methoxychlor, heptachlor, DDT, malathion, pentachlorophenol) were performed using the disc-agar diffusion method and recording effects on growth of isolates streaked on MSB agar containing glucose or succinate and yeast extract.

Protein determinations were performed according to the methods of Lowry et al. (1951).

RESULTS

Pure Culture Toxicity Studies

The toxicity of Kepone to a variety of pure cultures from our laboratory collection was determined by the disc agar diffusion sensitivity method. Table 1 shows the degree of toxicity as expressed by the size of the zone of inhibition. Only the sensitive cultures are shown. Several of the isolates (No.'s 1, 3, 4, 11, 14, 15, 29) were particularly sensitive (as judged by the size of the zone of inhibition) to Kepone. Chemical analysis has shown Kepone to be 99% pure with no trace (less than 0.5 ng/ml) of other chlorinated compounds. Of the 26 isolates tested, 33% were inhibited at the 3.65 μg/disc concentration and 50% were inhibited at the 14.6 μg/disc concentration. Fifteen percent of the isolates were inhibited at the 1.46 μg/disc concentration. Higher concentration of Kepone (20 μg/disc) inhibited 100% of the isolates.

Of the six fungal cultures tested, one was poisoned at the 14.6 μg/disc concentration. Two yeasts, _Candida maltosa_ and _Candida lipolytica_, were both sensitive but at higher concentrations of Kepone than used with the bacteria tested.

Kepone-sensitive cultures (i.e., showing zones of inhibition with disc assay) showed no significant correlation ($\alpha = 0.05$) with morphology, gram stain, hydrocarbon utilization, pesticide tolerance, amylolytic, lipolytic, and proteolytic activity, nitrate reduction, sugar utilization and urea hydrolysis. These results are summarized in Table 2.

Mixed Culture Toxicity Studies

To determine the toxicity of Kepone to mixed populations of bacteria from a variety of marine habitats, total viable counts were performed with Zobell's seawater agar (2-15) containing dissolved Kepone. Results of these assays (Table 3) are expressed as the percentage reduction in colony forming units (CFU) normalized against control plates that contained no Kepone. The results demonstrate that Kepone, in concentrations as low as 20 μg/ℓ, is inhibitory to the development of colonies on an agar plate. Different degrees of inhibition (concentrations of Kepone which would give the same reduction in CFU's) were noted with samples taken from the same area at different times. In many cases, concentrations below 20 μg/ℓ were inhibitory. Within each population, some microorganisms were quite resistant to Kepone (i.e., grew in its presence).

Twenty cultures, which grew in the presence of Kepone, were selected (based on predominance) for further study. The cell types and enzymatic activities of these purified isolates showed no significant correlation ($\alpha = 0.05$) with Kepone sensitivity.

Because Kepone is known to be present in sediments and detritus in concentrations higher than that found in the water column (U.S. Environ. Protection Agency, 1975), we attempted to determine the toxicity of Kepone to bacteria in sediments. The results of viable plate counts on Zobell's marine agar containing Kepone using samples taken from Range Point marsh sediment is shown in Table 4. Under aerobic conditions, CFU's decreased as a function of increasing Kepone concentrations as expected from earlier results. Under anaerobic conditions, however, the inhibitory effect of Kepone was reduced. For example, at 0.2 mg Kepone/l, a slight reduction (0 - 12%) in CFU's occurred under anaerobic conditions but a larger reduction (34 - 80%) occurred under anaerobic conditions. Colonies originally grown aerobically, also showed decreased Kepone sensitivity under anaerobic conditions. Sediment samples taken at different times generally showed different degrees of inhibition at the various concentrations.

The effect of Kepone on the anaerobic microbial populations in contaminated James River sediment was different. These sediments contained 0.1 ppm Kepone (A.J. Wilson, ERL-GB) when collected (8/29/77). Results of viable plate counts under aerobic and anaerobic conditions are presented in Table 4. As opposed to Range Point sediment, the James River sediments contained bacteria which did not show decreased sensitivity to Kepone under anaerobic conditions.

Oxygen Uptake Studies

To further assess the sensitivity of bacteria to Kepone, we conducted oxygen uptake studies. As seen in Table 5, oxygen uptake was a sensitive indicator of Kepone toxicity. The isolates showed varying degrees of sensitivity: most cell suspensions (10^6 - 10^7 cells/ml) were inhibited at the 20 mg/l concentration, whereas relatively little inhibition occurred at the 2 mg/l concentration. Inhibition at 20 mg/l, in many cases, was substantially greater than at the 200 mg/l (see isolates 32, 43, and 56). It should also be noted that some isolates actually increased oxygen uptake in the presence of Kepone (isolate 49).

DISCUSSION

Our results indicate that Kepone is toxic to microorganisms. Kepone concentrations as low as 0.02 mg/l reduced the number of colony forming units on Zobell's marine agar by 23-92 ^{o}/oo. This is ten to a hundred times more toxic than the PCB's (Bourquin 1975), chlordane (Trudgill et al. 1971) and heptachlor (Shamiyeh and Johnson 1973). Kepone levels in James River sediment have been shown to be 0.05 - 0.5 mg/kg, particularly in areas of high organic content (U.S. EPA, 1975). Since Kepone is resistant to chemical and biological degradation (Garnas et al. 1977) and since Kepone appears to be washing out of the James River system at a very slow rate, residual Kepone could have an inhibitory effect on bacteria in the river.

However, this compound was not universally toxic to all cultures at the concentrations tested. Since only certain members of the bacterial populations were inhibited by Kepone at concentration levels equal to those in the James River, it is important to know if the tolerant species will replace the sensitive species and thus maintain the metabolic integrity of the ecosystem. In general, this replacement process occurs in both soil (Tu and Miles 1976) and aquatic (Ware and Roan 1970) environments. Unpublished results from our laboratory, however, have shown that Kepone, at low concentrations, decreases the rate at which the pesticide methyl parathion is degraded, thus implicating a possible interference with the metabolic integrity of an aquatic system.

Of the three methods used to determine toxicity in this study (i.e. sensitivity disc, plate counts and oxygen uptake), each appear to yield similar toxicity results. Plate counts are the easiest method to assess toxic effects on a mixed population of bacteria, and the minimum inhibitory concentration (MIC = 0.02 mg/l) gives a good reflection of the effects possibly occurring under natural conditions. Little can be said about the MIC from agar-disc diffusion experiments except that low concentrations of Kepone are inhibitory.

Much higher concentrations of Kepone would appear to be necessary to inhibit oxygen uptake. However, these studies employed high densities of cells (10^6 - 10^7 cells/ml). These levels are, in fact, proportional to an enivornmental level. Escambia Bay waters have a cell density of 10^3 - 10^4 cells/ml. We have determined, in plate assays, that the growth of this population is inhibited at 20 μg/ℓ Kepone. By concentrating both factors 1000X, observe that 10^6 - 10^7 cells will require 200 mg Kepone/l to affect inhibition, which are the same levels employed in oxygen uptake studies.

The use of physiological characteristics (sugar fermentation, enzyme activities, urea hydrolysis, hydrocarbon oxidation, etc.) as a possible tool to determine the toxic effects on specific physiological processes has not been used extensively (Brown et al 1975a). Our study demonstrates no significant correlation between these characteristics and Kepone toxicity. Amylolytic activity was prevalent in some Kepone-sensitive organisms as was the case for in PCB-sensitive bacteria (Bourquin and Cassidy 1975). These effects apparently cannot promote the enrichment of amylase-positive organisms in a mixed population, because neither static nor flow-through systems exposed to Kepone in our laboratory showed this type of enrichment.

The nontoxic effect of Kepone on bacteria grown anaerobically points to a possible involvement with electron transport and respiration. Widdus et al. (1971) and Trudgill et al. (1971) have shown that the inhibition of NADH oxidase activity in Bacillus subtilis by chlordane occurred indirectly through the apparent disruption of membrane-mediated electron transport. Similar mechanisms could account for the affect of Kepone in anaerobic versus aerobic conditions. Trudgill et al. (1971) did not study the effect of chlordane on anaerobic growth, but he showed that a Streptococcus species (which would have minimal cytochrome-mediated electron transport activity) was unaffected by chlordane. Kepone, therefore, could interfere with membrane-mediated transport.

A possible explanation for the increased oxygen consumption in the presence of Kepone, is an oxygen uncoupling effect which would generate hydrogen peroxide. Hydrogen peroxide would then be split by catalase, releasing O_2 gas. The mechanism accounting for the release of oxygen by cells is still under investigation.

Table 1. Sensitivity of pure cultures to Kepone using the agar-disc diffusion method[a]

GBERL Isolate #	Acetone Control	Concentration Kepone (μg) per disc			
		1.46	3.65	7.3	14.6
1	0	0	+ + +	+ + + +	+ +
3	±	+	+ +	+ + +	+ + +
4	0	+	+ + +	+ + + +	+ + +
5	±	±	±	±	+
8	±	±	±	±	+
11	±	+ +	+ + +	+ + +	+ + +
14	0	0	+	+ +	+ + +
15	0	0	+ +	+ + +	+ + +
29	0	+	+ +	+ + +	+ +
36	±	±	+	+	+
42	±	+	±	+ +	+ + +
47	0	0	±	±	+
54	±	±	+ +	+ +	+ +

[a] 0 = no zone; ± = 0-1 mm; + = 1-2 mm; + + = 2-3 mm; + + + = 3-4 mm; + + + + = > 4 mm.

Table 2. Physiological activities of cultures used in Kepone toxicity study.

	Culture Number	Organism Type	Gram Stain	Alkane[a] Utilizer	Aromatic[a] Utilizer	Physiological Function[b] Lipo	Physiological Function[b] Amylo	Physiological Function[b] Proteo	Pesticide[a] Tolerance
Kepone Sensitive	1	rod	pos	+	+	-	+	+	+
	3	rod	pos	-	-	-	+	+	-
	4	cocc-bac	neg	-	-	+	+	+	-
	5	cocc	neg	-	-	+	+	+	-
	11	cocc-bac	pos	+	+	+	+	-	+
	14	rod	pos	-	-	+	+	-	-
	29	rod	pos	-	-	-	-	+	-
	42	coccus	pos	-	-	+	-	-	-
	47	cocc	neg	-	-	-	-	-	-
	54	rod	pos	-	+	-	+	+	-
	8	yeast	-	-	+	+	+	+	-
	36	yeast	-	+	-	+	+	+	-
	15	fungus	-	+	+	N.D.[c]	N.D.	N.D.	-
Kepone Insensitive	10	rod	neg	-	-	-	-	±	-
	18	pleo	neg	+	-	+	-	-	-
	20	rod	neg	+	-	N.D.[c]	N.D.	N.D.	-
	23	rod	pos	+	+	N.D.	N.D.	N.D.	+
	40	cocc-bac	pos	+	-	+	-	+	+
	45	rod	neg	-	-	+	-	+	-
	46	rod	neg	-	-	+	-	-	+
	56	cocc-bac	neg	-	-	+	+	-	+
	59	rod	neg	-	-	-	-	-	-
	2	yeast	-	+	-	-	-	+	-
	7	yeast	-	-	-	+	+	+	-
	27	fungus	-	-	+	N.D.	N.D.	N.D.	+
	44	fungus	-	-	-	N.D.	N.D.	N.D.	+

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[a] Hydrocarbons and pesticides listed in text

[b] Lipo = lipolytic activity; Amylo = amylolytic activity; Proteo = proteolytic activity

[c] N.D. = not determined

Table 3. Effects of Kepone on total viable counts of bacteria from estuarine water

Sample source	Date[a]	Salinity (^{o}/oo)	Temp. (C)	CFU's x 10^2 per ml water			
				Z-15	0.02[b]	0.20[b]	2.00[b]
Range Point	A	15	21	181	108(41)[c]	73(60)	35(81)
	B	15	24	86	65(25)	35(60)	33(61)
	C	20	23	1	12(28)	13(23)	13(25)
Escambia Bay	A	11	22	267	81(70)	233(13)	130(52)
	B	15	24	13	7(50)	10(26)	5(66)
	C	18	22	12	11(8)	9(23)	9(23)
Laboratory Pond	A	20	20	479	448(7)	409(14)	396(18)
	B	15	26	104	49(53)	-	19(82)
	C	20	27	544	501(8)	437(20)	309(43)
Gulf of Mexico	A	33	18	270	62(78)	32(89)	46(83)
	B	33	22	13	10(21)	10(25)	8(40)
	D	33	24	15	6(59)	1(92)	1(95)

[a] Sampling Dates. A = 4/10/77; B = 4/17/77; C = 4/29/77; D = 5/7/77.

[b] mg Kepone/ℓ used in Zobell's marine agar.

[c] Numbers in parentheses are percentage reduction in CFU's normalized against growth on control plates of Zobell's marine agar containing no Kepone.

Table 4. Typical Effects of Kepone on the production of colony forming units from Range Point marsh and James River sediments[a]

			CFU's x 10^2/gm sediment		
			Kepone (mg/ℓ)		
Sediment Source	Incubation conditions	Control Ƶ-15	0.02	0.2	2.0
Range Point	anaerobic	34	41(0)[c]	41(0)	37(0)
	aerobic	210	200(5)	140(34)	30(86)
James River	anaerobic	43	28(35)	24(44)	9(79)
	aerobic	15200	16500(0)	14200(7)	9400(36)

[a] James River sediment contained 0.1 ppm Kepone

[b] CFU's are the mean of the results of triplicate platings. Experimental error in these assays averaged ± 8 CFU's

[c] Numbers in parentheses are percent reduction in CFU's relative to Ƶ-15 control

Table 5. Effect of Kepone on oxygen uptake by resting cell suspensions of isolates from estuarine environments

Isolate no.	Source[c]	Kepone Enrichment Concentration	Percentage Reduction in O_2 Uptake[a]		
			Kepone (mg/ℓ)[b]		
			2	20	200
49	EB	0.00	+25	100	100
51	EB	0.02	0	100	100
61	EB	0.20	0	100	100
56	EB	2.00	66	75	66
32	RP	0.00	0	83	31
35	RP	0.02	11	100	100
43	RP	2.00	-	64	33

[a] nM O_2/ml/min/μg protein or glucose

[b] Kepone concentration required to show inhibition of concentrated cell suspension (10^6 - 10^7 cells/ml). One thousandth of this concentration would inhibit O_2 uptake by bacterial populations in Escambia Bay (10^3 - 10^4 cells/ml)

[c] EB = Escambia Bay, RP = Range Point

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Test of Model for Predicting Kepone Accumulation in Selected Estuarine Species

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Abstract

Extensive testing has shown that Kepone is rapidly accumulated by estuarine animals when administered in water or food. Flow-through laboratory experiments with oysters, shrimp, crabs, and fish indicate that food-chain transfer of Kepone is important in predicting Kepone residues in estuarine organisms. The rates of Kepone movement through estuarine organisms were previously unknown; rates of uptake and depuration by these organisms were determined with a regression model that describes mathematically the uptake and depuration of Kepone by these organisms. The model describes biological data as a single equation, thus allowing variations due to many physical, chemical, biological, and random error factors to be analyzed simultaneously.

The direct application of this model to cautious extrapolation will aid administrative decisions that affect water quality. The rates calculated by this single-species model also can be used in developing models that can predict the long-term fate of Kepone or other pollutants in an estuarine environment.

Key Words: Kepone, estuarine, food-chain, uptake (bioconcentration), model (mathematical).

Introduction

Kepone has been shown to accumulate and depurate from estuarine animals at rates that vary with the species and the source of contamination--water or food (Bahner et al. 1977). These laboratory-derived results complement field surveys by determining whether water, food, or both contribute significant quantities of Kepone to estuarine animals. However, variability in the laboratory and field survey data from the James River obscures trends and limits full utilization of these pesticide residue data.

Several reports are available that describe methods of analyzing laboratory derived fish residue data using kinetic models where exposure concentrations in water are known and closely regulated (Blau and Neely 1975, Branson et al. 1975). With minimal uptake data, these kinetic models can accurately predict equilibrium residues in fish, however, use of these models for invertebrate residue data has yet to be demonstrated.

In many instances, such as for field survey residue data, exposure concentrations, length of exposure, temperature, pH, and salinity are not known accurately and often several representative invertebrate and vertebrate species are sampled or tested. Field-exposed animals can depurate in a clean environment and time-series analyses of these residues are valuable in determining how long biota will retain a chemical. There is a need to analyze these field and laboratory uptake and depuration data, so that differences in exposure concentration, differences due to species or size-class, or route of uptake can be statistically examined. Therefore, a generalized mathematical equation was developed that: 1) describes the available data and delineates rates; 2) describes uptake from water data; 3) describes uptake from food data; 4) describes uptake singly; 5) describes depuration singly; 6) describes uptake and depuration simultaneously; 7) is expandable to multi-dimensions (describes uptake and depuration simultaneously by a species exposed to several concentrations, or describes uptake and depuration simultaneously by one or several species exposed to one or several pollutants); and 8) is one equation. This uptake/depuration equation can be incorporated into a stochastic, dynamic estuarine ecosystem model to assess movement of Kepone in James River biota.

Methods

Model-Building Process

Initial uptake of persistent chemicals by estuarine animals is normally rapid, but net accumulation diminishes until a constant concentration is reached (Fig. 1a). On the other hand, depuration of chemicals does not follow a consistent pattern, and rate of loss is dependent upon the nature of the chemical and the test species. There is variability in most experimental data due to fluctuation in physical, chemical, and biological factors such as temperature, chemical concentration, animal size, molt cycle, and seasonal spawning cycles. Chemical concentrations in water and food (the variables most critical in determining uptake/depuration) are easily controlled in the laboratory, however, molting and spawning cycle variations are difficult to control, and often are affected by a pollutant's toxic properties. We developed a statistical model to analyze mathematically the uptake of pollutant chemicals by estuarine animals in laboratory or field exposures. This method of analysis aids comparisons of laboratory with field data and permits use in larger ecosystem models to predict movement of pollutants in estuarine biota.

A nonlinear statistical model was designed (Daniel and Wood, 1971) to describe the uptake and depuration of chemicals because: 1) transformations of the residue data failed to provide an acceptable model that was linear in the parameters, and 2) one equation was desired to describe simultaneously both uptake and depuration since these are not mutually exclusive events. Effects, such as sublethal toxicity, should be distinguishable by statistically significant changes in parameter values of this model.

The linear equation, $Y=a+bX$, would describe uptake of pollutants if uptake were a linear relationship. Y would be the pollutant residue at time = X, and a and b would be the intercept and slope parameters to be calculated by the regression analysis. In a similar manner, the pollutant residue, Y, and time, DAY, are used by a non-linear regression procedure to calculate parameters of our non-linear uptake/depuration equation.

The general form of our model found to best fit residue data is: (1) $Y = [A + 10^{(-C \times DAY)}]^{-1} - [F + G \times e^{(-D \times (DAY - E))}]^{-1}$, where A, C, D, F, and G are parameters estimated with the natural logarithm of pesticide residue, Y, found at time, DAY. The effects of parameters A and C are graphically illustrated (Fig. 1a). Parameter A has the value of the uptake curve. The asymptotic residue concentration (in the animal at the end of exposure), RESIDUE, is determined by:

$\ln(\mathrm{RESIDUE}) = 1/A$ or $\mathrm{RESIDUE} = e^{(1/A)}$.

Parameter D (Fig. 1b) determines the slope of depuration, where $D \leq 0$ indicates no depuration and $0 < D < .7$ indicates increasing rapidity of depuration. E is not estimated from the data but is fixed at DAY = 'beginning of depuration'. In extreme cases, parameters F and G are required to modify the shape of the curve so the model will describe the data with greater accuracy. We found that parameters F and G usually can be replaced by A and 10, respectively, yielding the model:

(2) $Y = [A + 10^{(-C \times \mathrm{DAY})}]^{-1} - [A + 10 \times e^{(-D \times (\mathrm{DAY} - E))}]^{-1}$.

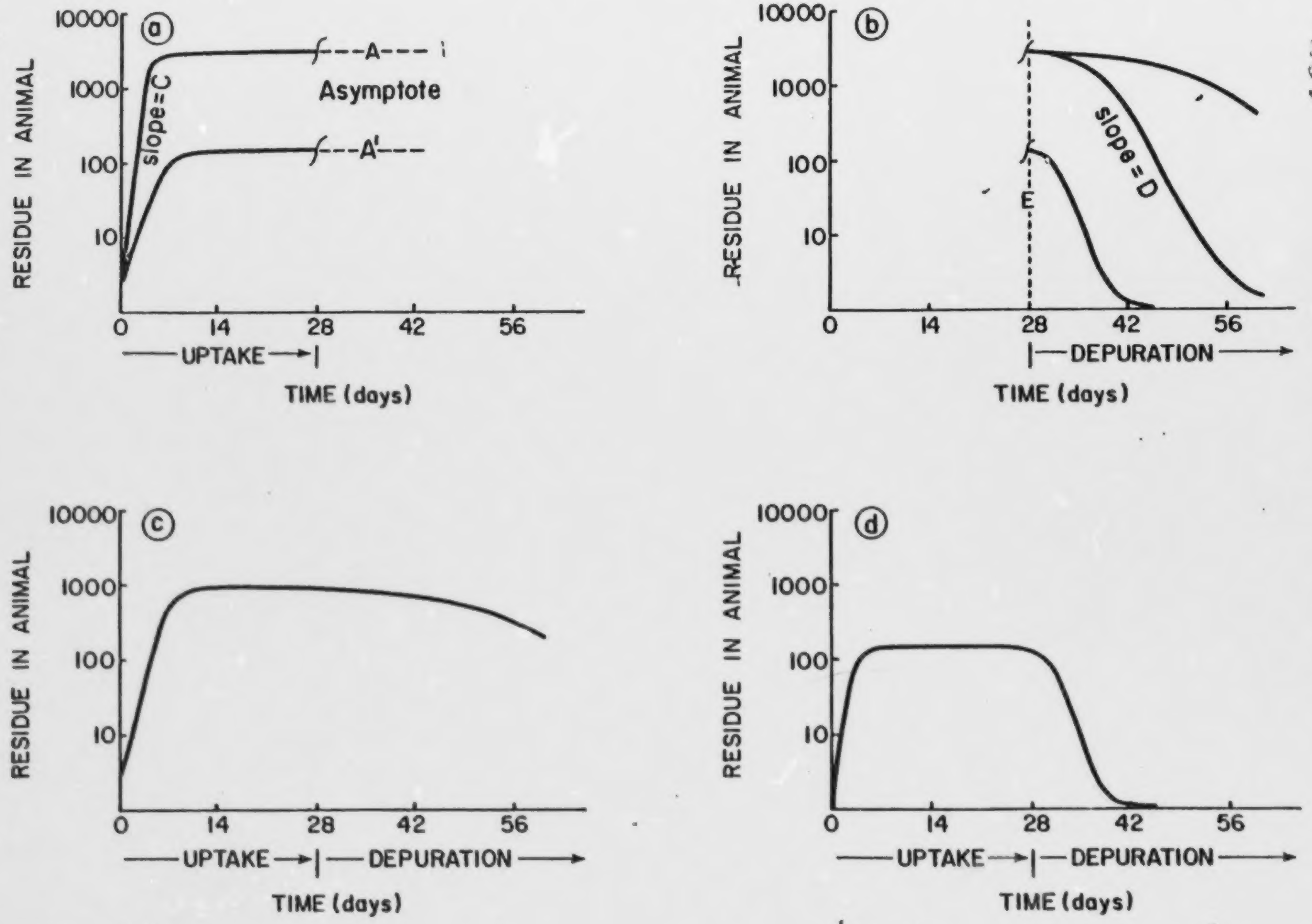
Results of this equation are shown graphically to indicate applicability to depuration data of different rates (Fig. 1c, slow depuration; Fig. 1d, rapid depuration).

Figure 1. (a) Plot of generalized uptake equation. Parameter A (A=0.125) in this illustration denotes upper asymptote; parameter A' illustrates effect of increase in value of A to 0.2. Parameter C determines initial uptake slope; normally, C varies from 0.1 (slow) to 2.0 (rapid).

(b) Plot of generalized depuration equation: parameter D determines loss slope and varies from 0.1 (top), 0.5 (mid), to 0.65 (lower); parameter E denotes onset of depuration.

(c) Plot of generalized equation, indicating slow depuration.

(d) Plot of generalized equation, indicating rapid depuration.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Both models (1) and (2), are nonlinear in the parameters and require an iterative method to estimate those parameters. The method of numerical analysis developed by Marquardt (1963) was chosen primarily because of its wide acceptance and general use. A typical program dataset (exclusive of job control), using the S.A.S. NLIN[1] procedure, follows:

```
DATA KEPONE; INPUT SPECIES $ CONC TISSUE $ RESIDUE DAY;
IF RESIDUE EQ 0 THEN Y = 0;
IF RESIDUE NE 0 THEN Y = LOG (1000*RESIDUE);
CARDS;
SPOT    .4  FILLET 0.00   0
SPOT    .4  FILLET 0.50   7
SPOT    .4  FILLET 0.86  15
SPOT    .4  FILLET 0.99  30
SPOT    .4  FILLET 0.87  37
SPOT    .4  FILLET 0.57  44
SPOT    .4  FILLET 0.38  54
SHRIMP  .023 WHOLE 0.000  0
SHRIMP  .023 WHOLE 0.038  7
SHRIMP  .023 WHOLE 0.072  9
SHRIMP  .023 WHOLE 0.088 14
SHRIMP  .023 WHOLE 0.120 21
SHRIMP  .023 WHOLE 0.087 28
SHRIMP  .023 WHOLE 0.100 30
SHRIMP  .023 WHOLE 0.100 35
SHRIMP  .023 WHOLE 0.084 42
SHRIMP  .023 WHOLE 0.055 49
SHRIMP  .023 WHOLE 0.023 56
PROC SORT; BY SPECIES CONC TISSUE;
PROC PRINT; BY SPECIES CONC TISSUE;
PROC NLIN BEST = 05 ITER = 200 METHOD = MARQUARDT; BY SPECIES CONC
TISSUE;
PARMS A = 0.1 TO 0.3 by 0.1
      C = 0.1 TO 0.7 by 0.2
      D = -.1 TO 0.3 by 0.2;
Q = (A+(10**(-C*DAY))); E = 28;
R = (A+10**EXP(-D*(DAY-E)));
MODEL Y = Q**(-1) - R**(-1);
DER.A = -(Q**(-2)) + R**(-1);
DER.C = (Q**(-2))*(10**(-C*DAY))*DAY*LOG(10);
DER.D = -10*(DAY-E)*EXP(-D*(DAY-E))*R**(-2);
_WEIGHT_ = DAY;
```

---

[1] Statistical Analysis System, SAS Institute Inc., Raleigh, N.C. Mention of commercial products does not necessarily constitute endorsement by the U.S. Environmental Protection Agency.

Results and Discussion

Our uptake/depuration statistical models (Equations 1 and 2) were validated using raw data from uptake and depuration of di-2-ethylhexyl phthalate by fathead minnows (Pimphales promelas) (Branson, Blau, and Mayer 1977). Nine sample periods were represented with four replicate single-fish samples for the seven concentrations tested. The parameter estimates for each concentration tested were generated from the five-parameter (Equation 1) and three-parameter (Equation 2) model equations; standard linear lack-of-fit tests were applied using the calculated parameters and no lack-of-fit was evident (Table 1). Bioconcentration factors (BCF), the concentration in animal at chemical equilibrium ÷ concentration in exposure water, were calculated using our statistical models. The BCFs from our models and the Dow Chemical BIOFAC model (Blau et al. 1975) are listed for comparison.

Bioconcentration factors and bioaccumulation factors (BAF, concentration in animal at chemical equilibrium ÷ concentration in exposure food) are shown for six species of estuarine animals exposed to Kepone in water, food, or both (Table 1). Uptake-only and depuration-only data were analyzed using the appropriate portions of

Table 1. Parameter estimates for five-parameter model[a] or three-parameter model[b] that describe uptake and depuration of di-2-ethylhexyl phthalate by fathead minnows and uptake and depuration of Kepone for six species of estuarine animals exposed to Kepone in water, food, or both.

Speties	Exposure Concentration (μg/ℓ or μg/kg)	Route of Uptake	Parameters						Model BCF[d] or BAF	Biofac[e] BCF
			A	C	D	E	F	G		
fathead[a] minnow	1.9	fresh water	0.141	1.392	.702	56	.751	76.820	630	774
"	2.5	"	0.133	1.455	.202	56	.504	6.153	733	915
"	4.6	"	0.124	1.416	.261	56	.564	11.911	680	838
"	8.2	"	0.125	1.763	.238	56	.674	11.079	371	467
"	14.0	"	0.120	1.741	.688	56	.773	165.856	304	364
"	30.0	"	0.114	1.699	.703	56	.752	73.976	220	252
"[b]	62.0	"	0.110	1.680	.074	56	-	-	141	204
oyster[b]	.03	sea water	0.184	1.099	.621	28	-	-	7641	
oyster[a]	.39	"	0.122	5.136	.303	28	.194	1.083	9305	
mysids[c]	.026	sea water	0.197	.293	-	-	-	-	6160	
"	.41	"	0.118	.465	-	-	-	-	11686	
grass shrimp[b]	.023	sea water	0.212	.183	.0980	28	-	-	4862	
"	.40	"	0.124	.220	.0994	28	-	-	7949	
"	Lafayette R.	unknown	0.155	1.	.0827	-	-	-	-	
blue crab[b]	.03	sea water	no detectable uptake							
"	.3	"	"	"	"					
"	250.0	food	0.211	.136	-.0571	28	-	-	.457	
"	250.03	food + water	0.217	2.624	-.125	28	-	-	.401	
"	250.30	" "	0.202	.123	-.0324	28	-	-	.564	
sheepshead[b] minnows	.05	sea water	0.177	.354	-.0561	28	-	-	5684	
spot[b]	.029	sea water	0.216	.168	.0923	30	-	-	3534	
"	.40	"	0.144	.304	.0850	30	-	-	2593	

[a] Y = 1/((A+10**(-C*DAY)) - (F + G*EXP(-D*(DAY-E)))); five parameter model.

[b] Y = 1/((A+10**(-C*DAY)) - (A+10*EXP(-D*(DAY-E)))); three parameter model.

[c] Y = 1 (A+10**(-C*DAY)); uptake only.

[d] BCF=Bioconcentration Factor = $e^{(1/A)}$ / (conc. in water); BAF = Bioaccumulation Factor = $e^{(1/A)}$/ (conc. in food).

[e] Branson, Blau, and Mayer 1977.

the three-parameter model (Table 1). The three-parameter model (Equation 2) was also used to describe the laboratory depuration of Kepone from grass shrimp collected in the Lafayette River, near Norfolk, Virginia. Application of this model to depuration-only data gave excellent results, indicating that data from field-exposed animals can be used to derive all parameter estimates except C, the uptake rate parameter; C was arbitrarily set to 1.0 for these data (C should be chosen so that $10^{(-C*DAY)}$ is ≤ 0.00001).

Parameter estimates for uptake and depuration of Kepone in six species of estuarine animals were calculated with our five-parameter (Equation 1) and three-parameter (Equation 2) statistical models (Table 1). The three-parameter model (Equation 2) sufficiently described (by graphical analysis) all uptake from water data except that for oysters exposed to 0.39 µg Kepone/ℓ. In this instance, the five-parameter (Equation 1) model was necessary for generation of adequate parameter value estimates. The three-parameter model was also sufficient to describe the uptake and retention of Kepone in blue crabs that were fed 0.25 µg Kepone/g, regardless of whether 0.03 or 0.3 µg Kepone/ℓ seawater were also administered to the crabs. Graphs of the computed models and data for several of the species tested are shown (Figs. 2-6).

Fig. 2. Model representation of bioconcentration of Kepone from water containing average measured concentrations of 0.03 or 0.39 µg/ℓ by oysters (Crassostrea virginica) exposed for 28 days, and its depuration by oysters placed in Kepone-free water for 28 days. Parameter estimates for oysters exposed to 0.39 µg/ℓ were: A=0.122, C=5.136, D=.303, F=.194, and G=1.083. For oysters exposed to 0.03 µg/ℓ, A=0.184, C=1.099, and D=.621. Lower limit of analytical detection was 10 µg/g.

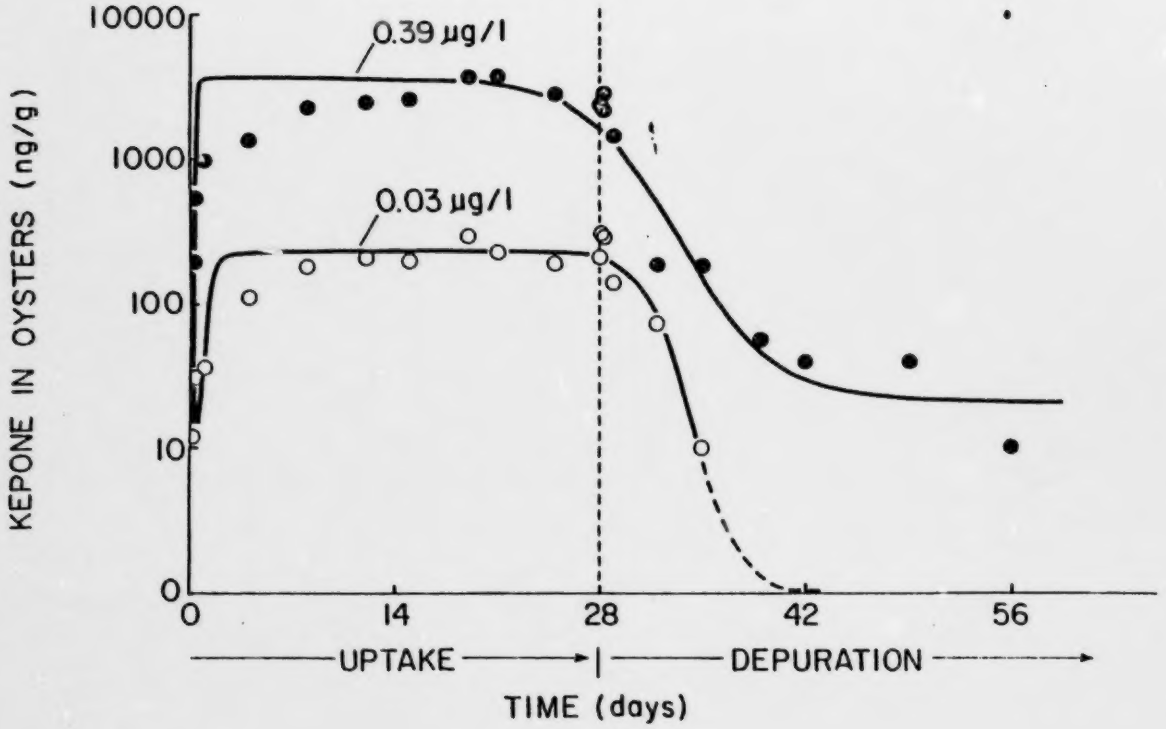

KEPONE IN OYSTERS (ng/g)
10000
1000
100
10
0
0.39 µg/l
0.03 µg/l
0
14
28
42
56
UPTAKE
DEPURATION
TIME (days)

Fig. 3. Model representation of bioconcentration of Kepone from water containing average measured concentrations of 0.026 or 0.41 μg/ℓ by mysids (*Mysidopsis bahia*) exposed for 21 days. Parameter estimates for mysids exposed to 0.026 μg/ℓ were: A = 0.197 and C = .293. For mysids exposed to 0.41 μg/ℓ, A = 0.118 and C = .465. Lower limit of analytical detection was 20 ng/g.

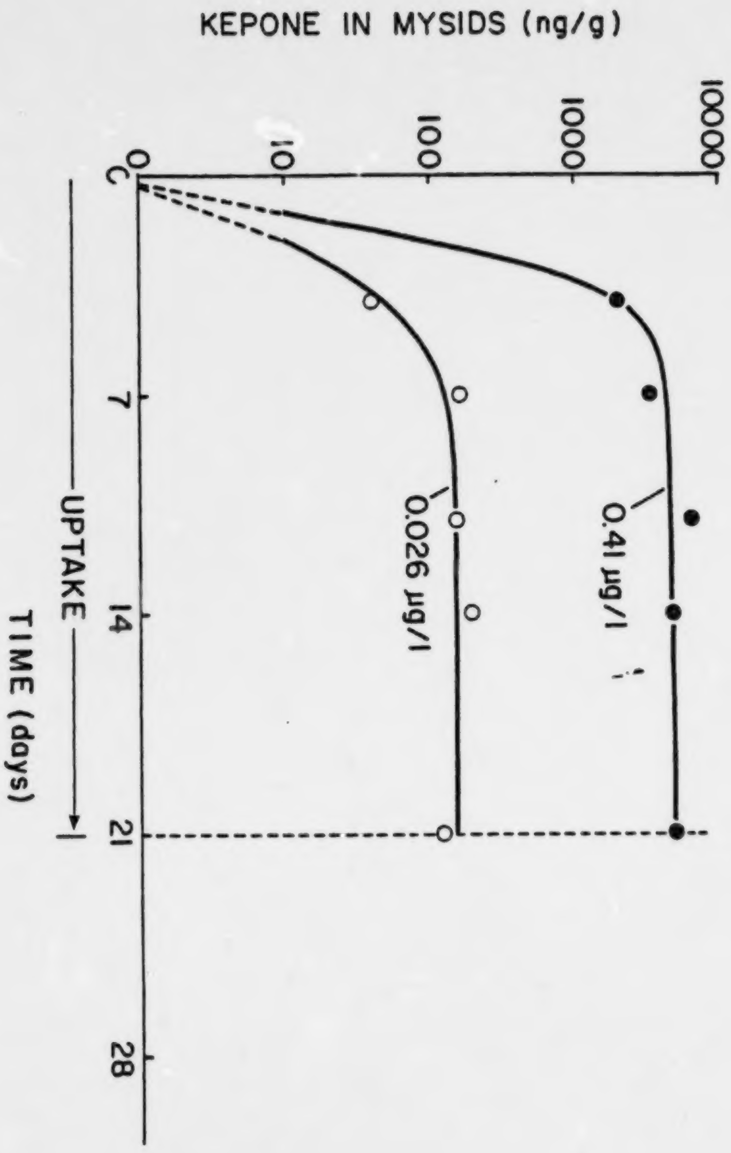

KEPONE IN MYSIDS (ng/g)
10000
1000
100
10
0
0
7
14
21
28
0.41 µg/l
0.026 µg/l
UPTAKE
TIME (days)

Fig. 4. Model representation of bioconcentration of Kepone from water containing average measured concentrations of 0.023 or 0.40 μg/ℓ by grass shrimp (*Palaemonetes pugio*) exposed for 28 days, and its depuration by shrimp placed in Kepone-free water for 28 days. Intermediate curve represents depuration of Kepone from grass shrimp collected in the Lafayette River, Norfolk, Virginia, and held in Kepone-free seawater at the ERL, Gulf Breeze, for 21 days. Parameter estimates for shrimp exposed to 0.023 μg/ℓ were: A=0.212, C=.183, and D=.098. For shrimp exposed to 0.40 μg/ℓ, A=0.124, C=.22, and D=.099. For Lafayette River shrimp, A=0.155 and D=0.083. Lower limit of analytical detection was 20 ng/g.

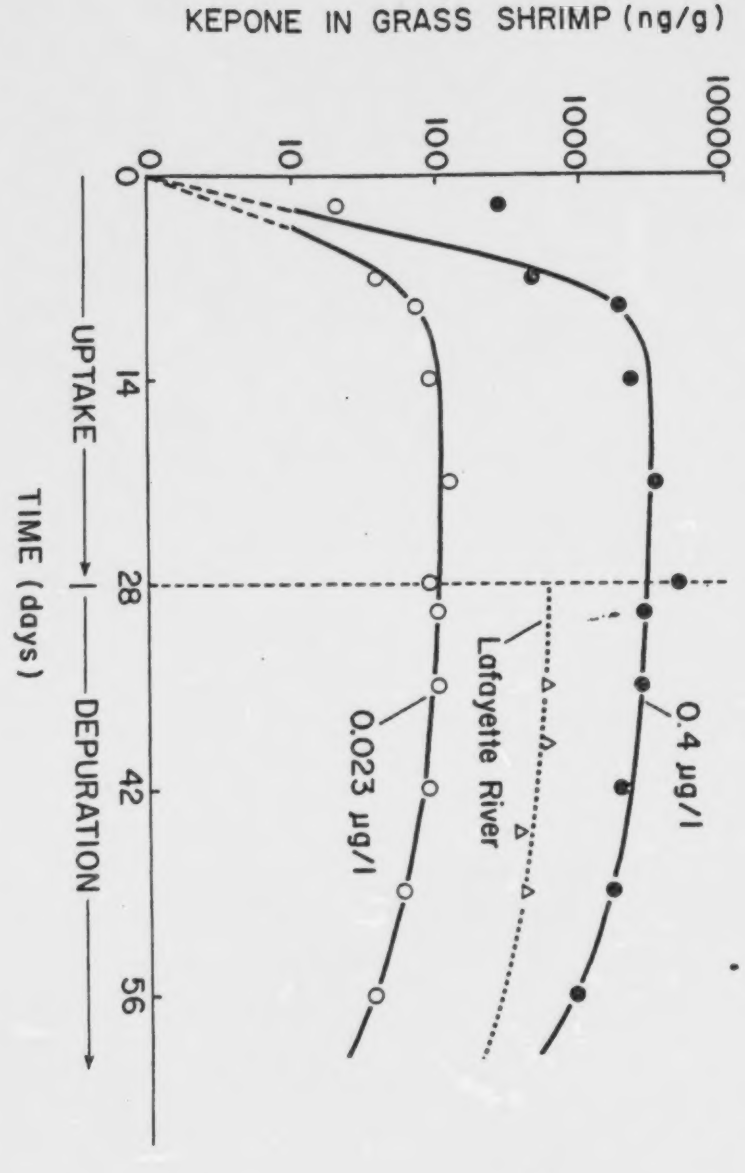

KEPONE IN GRASS SHRIMP (ng/g)
10000
1000
100
10
0
0
14
28
42
56
0.4 µg/l
Lafayette River
0.023 µg/l
UPTAKE
TIME (days)
DEPURATION

Fig. 5. Model representation of bioaccumulation of Kepone from food and water by blue crabs (Callinectes sapidus) exposed for 28 days followed by 28 days of consuming food and water containing no Kepone. Parameter estimates for crabs exposed to 250 ng/g were: A=0.211, C=.136, and D=-.057. For crabs exposed to 250 ng/g plus 0.3 μg/ℓ, A=0.202, C=.123, and D=-.032. Lower limit of analytical detection was 10 ng/g.

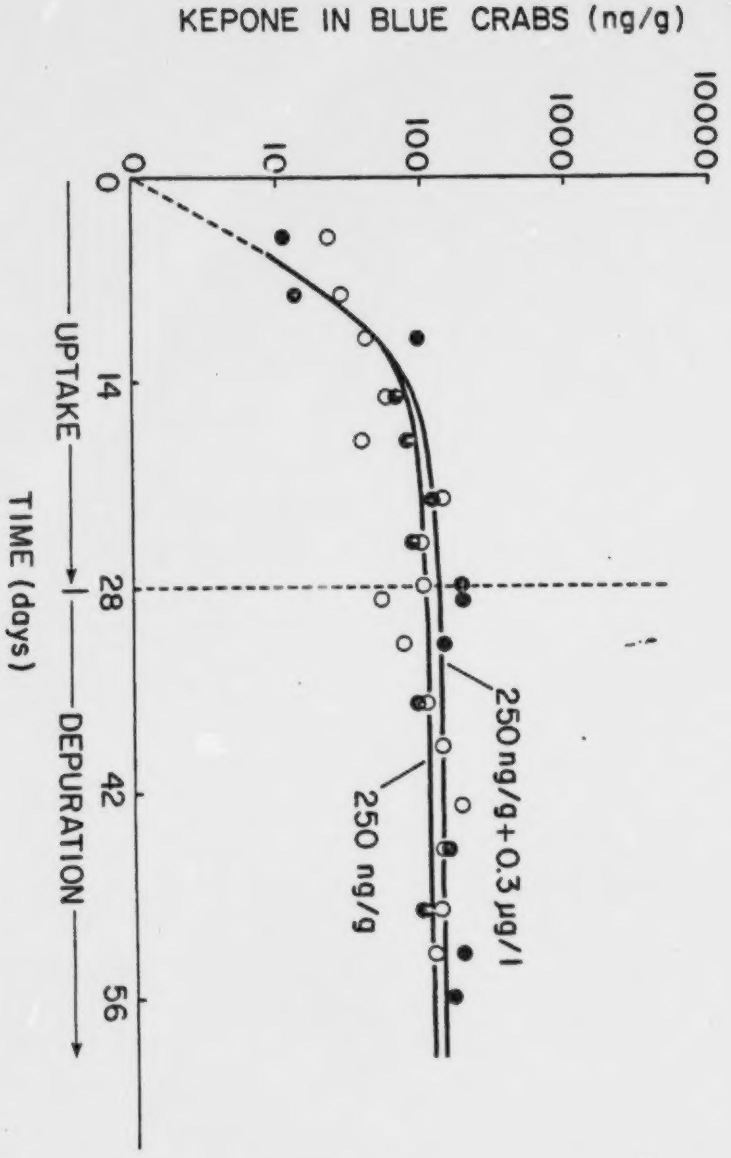
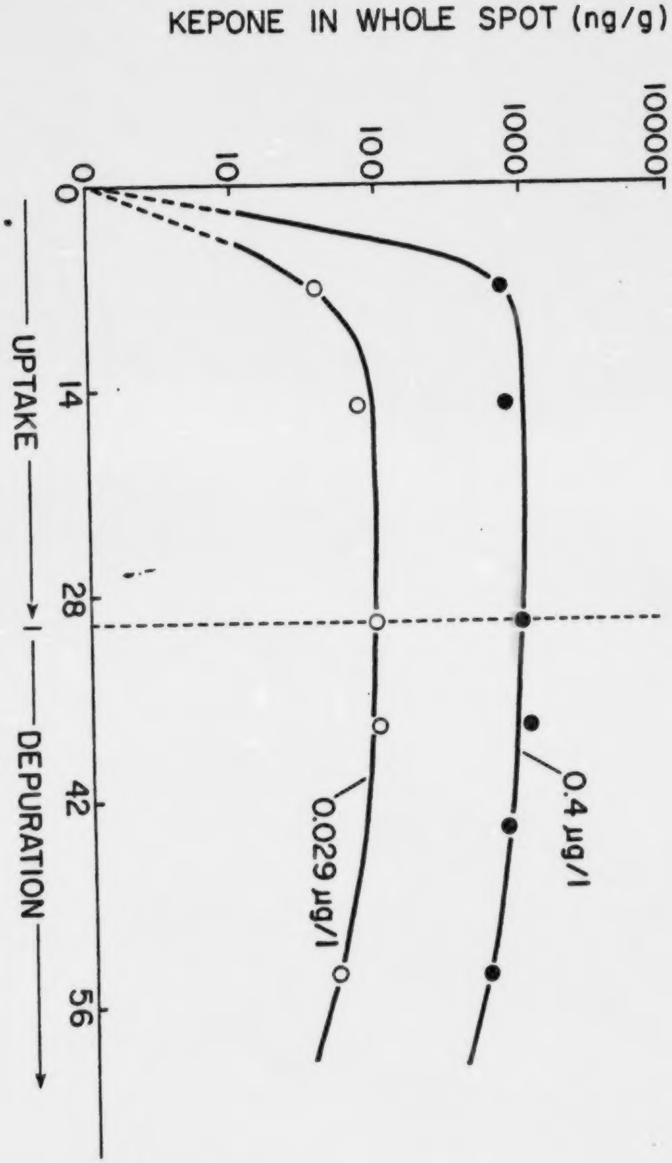

KEPONE IN BLUE CRABS (ng/g)
10000
1000
100
10
0
0
14
28
42
56
TIME (days)
UPTAKE
DEPURATION
250 ng/g + 0.3 µg/l
250 ng/g

Fig. 6. Model representation of bioconcentration of Kepone from water containing average measured concentrations of 0.029 or 0.4 μg/ℓ by spot (*Leiostomus xanthurus*) exposed for 30 days, and its depuration by fish placed in Kepone-free water for 24 days. Parameter estimates for spot exposed to 0.029 μg/ℓ were: A=0.216, C=.168, and D=0.092. For spot exposed to 0.4 μg/ℓ, A=0.144, C=.304, and D=.085. Lower limit of analytical detection was 20 ng/g.


KEPONE IN WHOLE SPOT (ng/g)
10000
1000
100
10
0
0
14
28
42
56
UPTAKE
TIME (days)
1
DEPURATION
0.4 µg/l
0.029 µg/l

Extrapolation from Model

Assuming the parameter values generated sufficiently describe the data, extrapolations to the time required for grass shrimp or spot to depurate 90% of their Kepone burden can be made. By applying the parameter values (Table 1) to the three-parameter model, 39 or 35 days are required for grass shrimp exposed to 0.023 or 0.4 μg/ℓ, respectively, to depurate 90% of their Kepone burden and 43 days for grass shrimp collected from the Lafayette River. Spot would require 41 or 42 days to depurate 90% of their Kepone residues if previously exposed to 0.029 or 0.4 μg Kepone/ℓ, respectively.

Extrapolation was not necessary for the oyster data, since non-detectable concentrations were reached. Insufficient data exist to determine the time needed for sheepshead minnows to depurate to nondetectable concentrations. Data and the model demonstrate that Kepone was not depurated from blue crabs.

Conclusions

The stochastic uptake/depuration model sufficiently described uptake and depuration of Kepone by estuarine oysters, shrimp, crabs, and fish, regardless of whether the pesticide was in water or in food. In addition, the model described depuration-only data, so that field-exposed animals could be utilized to project depuration rates and to allow estimation of the time required for residues to reduce to non-detectable concentrations. The ultimate residue concentration in exposed animals is predicted to depurate to zero concentration, if parameter D of the three-parameter model is greater than zero and given that time is sufficient. The exponential depuration portion of the model gives an accurate fit to data regardless of depuration rate, and given sufficient data, extrapolations can be made cautiously. All parameters in the model have confidence intervals; therefore statistical inferences (statistical significance) can be judged with joint confidence region comparisons. For example, the model can be used to determine if pesticide residues from laboratory exposures are statistically different than those from field exposures.

If expanded, the present single-species, single-concentration model should produce multidimensional models that describe chemical-species-dose-time interactions. Expansion of the number of parameters in the model will be limited by the number of replicated data points that are available. Because thorough, replicated sampling schemes testing multiple concentrations are not possible for all pollutants, attempts should be made to categorize parameter estimates for chemicals, species, and exposure concentrations. Successful categorization of the model parameters would allow use of generalized parameter estimates in dynamic ecosystem models.

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The Fate of ^{14}C-Kepone in Estuarine Microcosms

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Kepone was developed by the Allied Chemical Corporation in the early 1950's and was manufactured in Hopewell, Virginia intermittently until 1974. In 1973 Allied Chemical subcontracted the production of Kepone to Life Science Products Company, which operated from a building located adjacent to Allied Chemical's plant on the James River, which empties into Chesapeake Bay.

Their plant on the James River operated 24 hours a day, seven days a week, and manufactured 3,000 to 6,000 pounds of Kepone per day. Over 90% of the 1.7 million pounds of Kepone produced by Life Science during its 16 months of active operation was exported to Latin America, Europe, and Africa for control of insect pests. The effluents from the plant were hooked directly to Hopewell's sewage treatment plant. Kepone in the effluents disrupted the normal biological treatment process and put the plant out of operation. Later investigations revealed that sediment from adjacent Bailey's Creek and waste water from the sewage treatment and landfill areas contained Kepone at levels between 0.1 and 10 ppm. Sludge samples taken from the holding pond and from the landfill near the Hopewell sewage treatment plant contained 200-600 ppm of Kepone.

In August 1975 the Life Science plant was closed. EPA collected monitoring data showing widespread dissemination of Kepone in the James River system. Kepone in the water was detected at levels between 0.1 and 4 ppb; residues in fish and shellfish were between 0.1 and 20 ppm. The Food and Drug Administration set the allowable limit of Kepone in fish and shellfish consumed as food at 0.1 ppm. The James River was closed to fishing in December 1975.

In October 1976, Allied Chemical Corporation was fined $13.2 million for its role in polluting the James River with Kepone. Life Science Products Company itself was fined $3.8 million. It is now estimated by the scientists constituting the Kepone Task Force that approximately 200,000 pounds of Kepone have been disseminated into the James River system.

SLIDE ONE: Conceptual Fate of Kepone

Following the contamination of the James River system with Kepone, the Environmental Research Laboratory at Gulf Breeze responded with necessary data about the toxicity of Kepone to estuarine organisms and its potential for bioaccumulation and biomagnification. Serious questions arose concerning the fate of Kepone in the river. The fate of a pollutant is closely related to its toxicity; forces such as volatilization, sorption, metabolism, and abiotic transformation (photolysis, hydrolysis, chelation) affect the availability and toxicology of pollutants to aquatic species. A knowledge of the sites of Kepone concentration and rates of exchange associated with these sites is necessary for long term regulatory actions. Sorption and transformation data are needed to determine whether the ecosystem can remove the pollutant by degradation or eventual washout, or whether physical assistance from dredging or damming is necessary.

The conceptual fate of Kepone shown in this slide was developed and modeled by the Gulf Breeze Laboratory through a research project with Manhattan College. The projection of time required to reduce the levels of Kepone by various natural processes such as adsorption-desorption and transformation are included as an important phase of the project. Unfortunately, insufficient data are available for Kepone.

A variety of laboratory microcosms have been developed to supply fate information for this modeling effort. The following presentation is a brief summary of the research effort we conducted with Kepone in these systems.

SLIDE TWO: Kepone

Kepone is an extremely stable member of the cyclodiene insecticides, with a molecular structure similar to Mirex. However, due to the presence of an oxygen atom in the molecule, Kepone is more water-soluble through hydrogen bonding than Mirex. Within a pH range of 4-6, the solubility of Kepone ranges from 1.5 to 2.0 ppm. An increase of pH to 9-10 increases the solubility to 5-70 ppm. Although Kepone has a molecular configuration similar to Mirex, it should not be assumed to behave like Mirex in the environment.

SLIDE THREE: Static Fate System

This system consisted of a 125 ml Erlenmeyer flask fitted with a stopper. A capillary glass inlet allowed introduction of air or nitrogen; the gas exit was fitted to a disposable Pasteur pipette filled with XAD-4 resin (Rohm and Haas, Philadelphia, Pa.) to trap volatile compounds. The small size of the system allowed maximum replication and examination of different environmental substrates and processes. Similar systems were sampled sequentially with time to indicate rates of transport and transformation. ^{14}C-Kepone was used to minimize involved analysis and to facilitate simulation of environmental levels. The environmental parameters tested in this study were sediment type, salinity, aerobicity, pH, temperature, sunlight concentrations, volatilization, and biological forces.

Standard experimental conditions included 10 gm (wet weight) of sediment; 100 ml of Santa Rosa Sound water (18-24 ppth); constant temperature of 25°C; 12/12 hr. diurnal lighting (G.E. Vita Gro); water saturated air (Silent Giant); 19.9 μg ^{14}C-Kepone (1,941,000 dpm) added in 10 μl acetone carrier to water column; and duplicate systems sampled.

SLIDE FOUR: ^{14}C-Kepone Distribution in Static Fate System

In the standard analytical procedure, the system was fractionated into water suspendable particulate, and unsuspendable sediment (i.e., sand) by repeated rinsing of the system with equivalent salinity water; James River sediments were all suspendable (very little sand). Following centrifugation (3000 RPM), an aliquot of the water was examined for radioactivity by scintillation counting. The sediment fractions were extracted repeatedly with acetonitrile (Pe), with aliquots taken for scintillation. Following the addition of 2% sodium sulfate water to the acetonitrile extracts (4:1 water/solvent), the aqueous fractions were extracted with 1:1 petroleum ether/diethyl ether and analyzed by thin layer chromatography (3:1 diethyl ether/n-hexane; Quanta Gram LQGDF, Quanta Industries, Fairfield, New Jersey) and autoradiography (Birchover Radiochromatogram Spark Chamber, Hitchin, England). Periodically, the extracts were cleaned on florisil columns (2 gm, hexane washed; 20 ml rinse of 5% diethyl ether/hexane; final elution with 50 ml of 1% methanol/benzene) and analyzed for Kepone, octachloro-Kepone, and nonachloro-Kepone (standards provided by A. J. Wilson, EPA, Gulf Breeze, Florida) by electron capture gas chromatography (Hewlett-Packard GC Model 5830A; Ni^{63}detector; Model 18850A GC terminal; 2 mm id x 2 m glass, 2% OV-101 on Gas Chrom Q, 100/120). Extracted sediments (Pc) were dried and combusted at 900° (Harvey Instrument OX-200 Combustion System, Hillsdale, New Jersey) to liberate and trap residual radioactivity as $^{14}CO_2$.

The experimental results revealed that within one to two days, approximately 75 to 80% of the total radioactivity added to the system was found associated with the sediment. Most of this radioactivity (Pe) was adsorbed to the suspendable particulate material. Kepone, which could not be extracted from this material by organic solvent (Pc), constituted approximately 2 to 6%

of the total radioactivity. Very little Kepone was associated with the heavier sand material in the sediment. Virtually no Kepone volatilized out of the flasks (R) (no radioactivity in resin traps). The remainder of the radioactivity, that is 15 to 20% of the Kepone initially added, remained in the water column (W). In most experiments, total recovery of the radioactivity from the flask was between 90 and 95%. No evidence for the production of degradation products at any time was seen. Long-term incubations did not change this basic distribution of Kepone in the flask. When similar systems were sterilized with 2% formaldehyde or forced to become anaerobic by bubbling with nitrogen gas, a similar distribution of Kepone was apparent. Similarly, changing salinities, exposure to sunlight, varying concentrations of Kepone, and differing temperatures, all showed very little effect on this basic Kepone distribution pattern. Under all of these conditions, no evidence (total recovery of radioactivity as Kepone) was obtained for degradation of the pollutant.

In further tests with these screening systems, a sediment washing step was included to determine the desorption capability of Kepone from particulate material. This procedure consisted of washing Kepone-containing sediment three times with clean seawater. The washed sediments were then placed in a flask, covered with a column of fresh seawater, and incubated for three days. At the end of this incubation, the amount of radioactive Kepone in the water column was determined. This procedure was followed on test systems for the final three week sampling period. Typically, 9 to 11% of the radioactivity in the sediment desorbed into the water column in the first week wash. Another 4 to 8% of the radioactivity in sediment leached into the water column during each of the second and third week periods. Overall, 20 to 40% of the total radioactivity in the sediment was desorbed.

From these experiments, we conclude the following:

A. Kepone has a propensity for adsorption to and desorption from sediment. Most of the Kepone in the sediment was associated with the lighter suspendable particular matter and not with heavier sand particulate.

B. Kepone does not volatilize out of an aquatic ecosystem and there was no transformation or degradation of the Kepone.

C. The distribution of the Kepone between the water and the sediment (i.e., the Kp value), was not altered by changing environmental conditions such as aerobicity, temperature, Kepone concentrations, sunlight, and salinity.

SLIDE FIVE: Adsorption Isotherms for Kepone in Sediments

Adsorption isotherms are shown on this graph for various environmental and reference substrates. The adsorption data for all systems fitted linear isotherms over a broad range of water phase concentrations. The Kp's of Kepone show an increase with increasing organic carbon content from quartz sand (O.C. = 0.01%) to Range Point salt marsh detritus (O.C. = 25%). Quality of organic carbon, as well as quantity, influenced the Kp of Kepone. Ground seagrass (Thalassia), which was aged for several weeks in raw flowing seawater, displayed an organic carbon content of 60%. The Kp for Kepone with this substrate was lower than that for sediment from a local salt marsh with lower organic carbon content (O.C. = 25%). The presence of animal detrital matter in the saltmarsh sediment could account for this anomaly.

Field samples from the James River System (open and closed symbols) showed the same correlation with organic content of the sediments. With sediments of lower organic content, insufficient Kepone was present in the water to determine Kp values.

SLIDE SIX: Schematic of Continuous Flow System

Continuous-flow microcosm studies allow the dynamic nture of an ecosystem to be investigated in the laboratory. They provide information on the rate and extent to which a pollutant will a) move into the various compartments of an aquatic ecosystem and b) be transformed by biological and nonbiological forces.

The system consists of a reactor vessel containing the substrates and biota of interest. Raw seawater can be fed continuously to the system. The aeration apparatus samples exiting air for volatile organics and $^{14}CO_2$.

To follow the fate of pollutants resulting from the biological activity of aquatic macro biota, two continuous flow systems were structured with 9 cm of Range Point sediment and 24 liters of Santa Rosa Sound water. The systems were allowed to acclimate statically with aeration for 48 hrs. Twelve lugworms (Arenicola cristata) were added to one of the tanks and flow was started through both systems ($D = 0.04\ hr^{-1}$); after four days, the water flow was stopped and each system was spiked with 1 mg (1×10^8 dpm) of ^{14}C-Kepone in 0.5 ml acetone. After two days the water flow was again started. Aliquots of water (3 ml) were sampled directly for radioactivity until background levels were obtained. At that point larger sample volumes of water were required for detection of radioactivity. Thereafter, the radioactivity was concentrated on two beds of XAD-4 resin (75 ml wet volume in 250 ml separatory funnel). Following removal from a system, the resin was extracted with methanol in a Soxhlet extractor overnight and analyzed directly for radioactivity.

SLIDE SEVEN: Desorption Kinetics for Continuous Flow Systems

The resin was efficient in the removal of ^{14}C-Kepone from raw seawater (99%); the Soxhlet extraction procedure removed all radioactivity from the resin throughout these studies (95% recovery).

The graph shows the washout of radioactivity, plotted as a function of the radioactivity remaining in the sediment at any time. By Day 5 all of the lugworms were dead, with fungal mats forming over the tissues. One lugworm was removed from the system and found to contain high levels of radioactivity in its tissues (3% of total spike to system); all of the radioactivity was Kepone. At these levels of accumulation, the lugworms added to the system contained a large fraction of the original fortification (3% x 12 = 36%). The other dead lugworms were left in the system, where they eventually decayed and completely decomposed. Desorption followed simple first-order kinetics and gave a desorption rate of .002 hr^{-1} (that is, .002 of the original Kepone in the sediment desorbed per hour). This desorption rate was a reflection of the diffusion of Kepone off the sediment material and was, therefore, independent of flow rate. Numerous water samples were checked for the presence of particulate matter containing adsorbed radioactive Kepone. In all instances, of the radioactivity in the water, only 5 to 7% was associated with this particulate material and, therefore, washout was not a function sediment scouring. Thus, the radioactivity in the water was a true reflection of the dissolved Kepone present.

Since desorption of Kepone is probably a simple diffusion process, its ultimate distribution within the James River system will depend on the hydrodynamics of the river itself. However, ingestion of Kepone by a benthic organism could decrease the rate of desorption due to an additional sequestering compartment. These experiments indicate that the presence of polychaete worms in the sediments of the flow-through systems increased the amount of Kepone adsorbed into the sediments relative to worm-free control systems. This was presumably due to a bioaccumulation. In this particular experiment, Kepone was toxic to the polychaete worms and their eventual death and decomposition also resulted in a slower release of Kepone from the system. The slower rate of desorption is presumedly not due to increased amounts of organic material from the worm (not greater than 0.01% of the natural organic matter present), but is more likely a different form of sequestered Kepone that was desorbed slower. These data indicate that desorption of Kepone from sediment was a function of the quality of organic matter present and not the quantity. For additional sorption studies ^{14}C Kepone-containing water (0.1 ppm) was continuously passed over a sediment bed at a dilution rate = 0.01 hr^{-1}.

SLIDE EIGHT: Conclusions for Kepone Transport.

The adsorption capacity was large and would probably not reach saturation levels within reasonable time limits. The amount of Kepone adsorbed to the sediments in these experiments was a function of the rate at which the water passed over the sediment. Kepone adsorbed to the top 2-3 cm of sediment in these studies and little diffusion down the sediment column was observed. The removal of Kepone from inflowing water (60% at 0.01 hr^{-1}) was constant with time over 600 hours of flow. If the flow to the reactor vessel was stopped, preventing additional Kepone from entering the system, the residual Kepone in the water continued to be adsorbed rapidly into the sediment.

SLIDE NINE: Schematic of Intact Core System

The final system used to study the fate of Kepone in the James River estuary was a series of microcosms referred to as Eco-cores. These systems consist of glass tubes (30 mm in diameter and 38 cm long) which can be inserted into the water and into the underlying sediment layer to extract an intact core. These cores are then stoppered into the glass column, brought back to the laboratory, and tested for their biodegradation capacity. In the case of the James River, direct coring was not possible and thus sediment and water samples collected from the James River were mechanically placed in the glass columns to simulate a cored sample. In addition to the James River sediment studies, simultaneous cores were taken from a local salt-marsh near Pensacola Beach and studied in a similar manner. The advantage of these Eco-cores, besides their potential for looking at processes in intact core samples, is that they allow a large number of environmental parameters to be tested on a particular degradation process taking place within the cores. These degradation processes were monitored as follows: Once the core has been taken and brought back to the laboratory, the glass column was outfitted with an aeration device and the water in the core spiked with radioactive Kepone at a concentration of 500 ppb. The aeration of the water column disperses and mixes the Kepone and ensures contact with the sediment. These cores were then incubated at 25°C. Degradation was measured two ways: (a) Exiting gas from the cores was bubbled through alkaline solutions to trap radioactive carbon dioxide released from the radioactive Kepone; (b) Samples were taken from the water column and analyzed (by the same basic procedures used in the static system) to obtain the amount of Kepone remaining at any time and the presence and amount of any degradation products formed. After sufficient periods of incubation (i.e., diminished activity), the distribution of radioactivity in the cores was analyzed. Water and sediment were removed from the core and each component extracted and analyzed for the presence of radioactivity and the presence of any degradation products from the Kepone.

SLIDE TEN: Kepone Degradation Studies

SLIDE ELEVEN: Data from Coring System

A variety of conditions were varied in all of the systems described to observe the effect on the degradation of Kepone. Extremes in salinity, temperature and light did not alter Kepone. Analysis by GLC showed no detectable dechlorinated products of Kepone in any of the systems examined.

Since some chlorinated hydrocarbons are known to undergo dechlorination reactions under anaerobic conditions, cores were set up under a variety of environmental conditions. For example, cores were made anaerobic by bubbling with nitrogen gas; cores were made anaerobic and supplemented with glucose and other organic materials; and cores were maintained aerobically and supplemented with organic substrates which might stimulate the microbial degradation of Kepone. In all cases no evidence for Kepone degradation was obtained. In the analysis of several cores maintained under anaerobic conditions, thin layer chromatography revealed extraneous radioactive products which chromatographed separately from Kepone.

SLIDE TWELVE: Kepone Analysis

With anaerobic conditions in Eco-core experiments, thin layer autoradiography analysis revealed two radioactive spots, one of which was Kepone. Radioactive profiles obtained with high pressure liquid chromatography also showed similar radioactive distribution. When rechromatographed on TLC or HPLC the unknown radioactive spot migrated with the same Rf. In contrast, extracts of exposed lugworms displayed two radioactive areas; however, when rechromatographed, the unknown spot migrated at the Rf of Kepone. An isolate from estuarine sediments (corynebacterium-like) previously grown on minimal media and camphor as the sole carbon source, also displayed the unknown product. Mild acid treatment of the unknown would cause it to chromatograph like Kepone. The unknown spot failed to elicit a response on GLC-ECD at the Rt of Kepone and did not chromatograph favorably under most conditions. At this time the material obtained from the anaerobic sediment/water systems and the pure culture study are being examined in greater detail by GCMS and selective detectors.

CONCLUSIONS

At this time, we believe that Kepone does not degrade in our experimental systems. The reversible quality of the unknown back to Kepone causes us to suspect that a strong association is occurring from the presence of the oxygen atom in the molecule with other chemicals in the extracts and not degradation. Kepone did not volatilize from any of the systems described.

Kepone displayed a dynamic movement potential in sediment/water systems. The rate of this movement was related to the organic content of the sediment and to the quality of the organic fraction.

These systems were used to indicate the major environmental components affecting the fate of Kepone. Extrapolation of these data to the James River system is hampered by severe scaling problems. However, these studies do validate current field monitoring practices and offer direction for future field work to supplement the existing data base for mathematical modeling.

A BENTHIC BIOASSAY USING TIME-LAPSE PHOTOGRAPHY TO MEASURE THE EFFECT OF TOXICANTS ON THE FEEDING BEHAVIOR OF LUGWORMS (POLYCHAETA:ARENICOLIDAE)

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(Eds) W.S. and J.F. Vernberg. Academic Press. 1977. (in press)

ABSTRACT

A benthic bioassay was developed utilizing time-lapse photography to measure the feeding activity of a lugworm, Arenicola cristata. Automated 35 mm cameras were used to record formation of feeding funnels at 12-hour intervals. Substrate surface area reworked by lugworms held under identical conditions in separate aquaria was plotted against time to determine substrate reworking rates for each group. Rates were subjected to linear regression analysis and compared to demonstrate that no significant difference between the slopes of the calculated lines existed. Therefore, a difference in slope when one group is exposed to a toxicant could provide a measure of effect on lugworm activity. To demonstrate the applicability of this approach, lugworms were exposed to the pesticide, Kepone, and their rate of substrate reworking was compared with non-dosed lugworms. Kepone was acutely toxic to lugworms at a concentration of 29.5 μg/ℓ. A significant difference in substrate reworking rates was observed following exposure to concentrations as low as 2.8 μg/ℓ Kepone in seawater. It is suggested that a behavioral response to toxicity testing provides a sensitive and realistic approach for evaluation of ecological impact of pollutants on the marine environment.

INTRODUCTION

Federal legislation (i.e. the Marine Protection, Research and Sanctuaries Act of 1972) requires that permits for the discharge of materials into coastal waters be evaluated on the basis of their ecological impact on the marine environment. Under this legislative mandate the U.S. Environmental Protection Agency (EPA) has been delegated the responsibility to establish guidelines for conducting bioassays used to define the types and amounts of materials that may be released into the marine environment. The bioassay, therefore, serves as a regulatory tool used by federal agencies and private industry to assess the ecological impact of pollutants on the marine environment.

Bioassay procedures recommended by EPA (1976) for conducting toxicity evaluations utilize a variety of sensitive epibenthic and pelagic species but do not include representative infaunal organisms. This is due, in part, to the relative lack of sensitivity displayed by many infaunal species and the difficulty in observing biological effects while organisms are buried in sediment. However, many macrofaunal invertebrates are deposit feeders that have a great effect on the benthic community as a result of their substrate reworking activity. These organisms have been shown to influence benthic community trophic structure and sediment stability (Rhoads and Young 1971). In addition, sediment processing organisms provide a pathway for cycling organic material, nutrients and pollutants between the sediment and the water column (Rhoads 1973, Meyers 1977). Therefore, meaningful evaluations of the impact of a pollutant on the marine environment must include information regarding effects on representative members of the infaunal community. The objective of this study was to develop a sensitive and practical method that could be used to assess biological effects of pollutants on estuarine and marine infaunal organisms.

Various benthic organisms including holothurians, crustaceans, pelecypods and polychaetes, produce distinct, characteristic topographical features on the substrate surface as a result of their normal activity (i.e. feeding, burrowing and excretion). These features in some cases may serve as in situ indicators of the organisms' activity (Rowe et al. 1974). With the aid of time-lapse photography, surface features can be monitored and analyzed statistically to determine the relative effects of xenobiotics on organisms selected for study.

The benthic bioassay described here utilizes time-lapse photography to measure the formation of feeding funnels produced by the lugworm, Arenicola cristata Stimpson. Comparisons of rates of feeding funnel formation between exposed and control lugworms serve as the test criterion.

Ecological Significance

Lugworms are sedentate polychaetes distributed throughout the world in most littoral habitats. Their activities, which are somewhat analogous to those of the earthworm, are responsibile for bioturbations of the sediment to depths as great as 50 cm. Populations of the european species, Arenicola marina, have been observed to turn over nearly 500 tons of sand (dry weight) per acre per year (Blegvad 1914).

Lugworms form u-shaped burrows in a variety of substrates ranging from silt and mud to coarse gravel and mud. Arenicola cristata normally builds its burrows in muddy sand (partical size 200-700 μm) at depths of 20-25 cm and in densities as great as 20 per square meter (D'Asaro 1976). The burrow consists of a tail shaft, a horizontal gallery and a head shaft. Periodically, the lugworm moves forward in the gallery and ingests sand along with associated organic material (living and dead). The resulting displacement of the overlying sediment produces a subsiding column of sand marked by a funnel shaped depression on the substrate surface. When the organic content in the region of the head shaft is depleted, A. cristata forms new feeding funnels in adjacent areas. Figure 1 illustrates the progressive formation of feeding funnels produced by one lugworm at 12 hour intervals.

Feeding and the consequent formation of feeding funnels is an integral part of an activity sequence which also incorporates excretion and peristaltic pumping of water through the burrow for respiration and ventilation. These combined activities comprise the "Normal Cyclical Pattern" which is believed to be controlled by internal pacemakers and is therefore independent of normal environmental variables (Wells 1966). A decrease in the formation of feeding funnels would indicate an interruption in this activity cycle.

As the lugworm feeds and pumps water through its burrow, it mixes organic material and oxygenated water into the substrate (especially in the vicinity of the head shaft). This process is undoubtedly beneficial to other infaunal organisms living in association with the lugworm, for it provides them additional sources of food and oxygen. However, during periods of environmental perturbation, contaminating agents are also transported into the substrate. Garnas et al. (1977) demonstrated that lugworm activity affected movement of the pesticide methyl parathion from the water column into the sediment. The fate of such compounds as they interact with infaunal organisms raises questions of great complexity. The role of the lugworm in this regard is not yet fully understood. However, the lugworm has been observed to suspend substrate modifying activity when environmental stress reaches a threshold level (Rubinstein 1976). This type of behavioral response to environmental contaminants could serve as a sensitive indicator for various polluting agents.

Feeding Activity of Unstressed Lugworms

To insure that comparisons between exposed and non-exposed lugworms could be made, I conducted preliminary tests to determine if lugworms of similar size rework the substrate at equivalent rates when subjected to the same conditions. Due to a limitation in available time-lapse photography equipment, only two groups of lugworms could be compared at a given time.

Bioassay Procedure

Two 125 ℓ aquaria were used as test habitats. Both aquaria contained 0.25 m^2 of sand (particle size 200-700 µm) to a depth of 25 cm and 72 ℓ of 20 µm filtered seawater. All tests were conducted at salinities between 20 and 23 $^{o}/_{oo}$ and temperatures between 22 and 25°C. Water was aerated by airstones except when automatically turned off prior to taking photographs.

Lugworms were obtained from stocks cultured at the University of West Florida Marine Laboratory, Sabine Island, Pensacola, Florida by the method of D'Asaro (1976). Six worms 8.5 to 9.5 cm long (measured fully contracted) were placed in each aquarium and allowed 48 hours to establish burrows and acclimate to test conditions. Following acclimation, 50 grams of ground seagrass (predominantly Thallasia testudinum) was added to both aquaria. The seagrass formed a dark mat on the sediment surface and served as the detrital component of the benthic system. It was used as food by lugworms and also provided photographic contrast against the white underlying sand when turned under by feeding animals.

A 35-mm single lens reflex camera was positioned above each aquarium (Figure 2). The cameras were equipped with an automatic advance mechanism, 24-hour timers and an automatic lighting system consisting of four strobes and two floodlights. Photographs of the substrate surface were taken at 12-hour intervals for 72 hours and then analyzed to determine the surface area disturbed by feeding lugworms. Surface area was calculated by using a system of point counting in a coherent grid modified from Hyatt (1973). The outline of feeding funnels was traced onto a 0.25 cm grid overlay, all points on the 5 mm intersections were counted and then converted to actual surface area (cm^2). Total surface area turned under by feeding lugworms was plotted against time to determine the substrate reworking rate of lugworms in both aquaria. Rates were subjected to linear regression analysis and the slopes of the calculated lines were compared; differences due to treatment were considered significant at $\infty = 0.01$.

Six replicate tests were conducted with a different group of lugworms for each test. Results are shown in Figure 3. Variability in the magnitude of surface area disturbed between tests can be attributed to slight variations in size, age and condition of lugworms. For this reason comparisons of substrate reworking rates were made within a test and not between separate tests. Rates of feeding funnel formation were not significantly different (t[97.5; 8]) between aquaria within each of the six tests. Therefore, the use of two aquaria, one experimental and one control is valid because the rates of substrate reworking between lugworms of similar size and condition are comparable.

Application of Feeding Activity To Toxicity Testing

Following preliminary tests using no toxicant the sensitivity of lugworm feeding activity was tested with the insecticide Kepone (dodecachlorooctahydro-1, 3, 4-metheno-2H-cyclobuta [cd] pentalene). Kepone was selected because of the extreme hazard it poses to aquatic life in the James River Estuary and Chesapeake Bay (Hansen et al. 1977), areas in which the lugworm is endemic. Although the acute toxicity of Kepone to several estuarine fishes and invertebrates has been investigated (Hansen et al. 1977, Schimmel and Wilson 1977, Nimmo et al. 1977) the sub-lethal effects of this compound on infaunal organisms have not been evaluated.

METHODS

A series of six tests using diminishing concentrations of Kepone was conducted using the bioassay procedure previously described. A stock solution of Kepone (88% pure) in nanograde acetone was dispersed in the water of one aquarium; the second aquarium received an equivalent amount of the acetone carrier and served as the control. Aquaria were dosed following lugworm acclimation and approximately one hour after the ground seagrass was added. From this point on photographs of the substrate surface were taken at 12-hour intervals for 144 hours. Measured concentrations of Kepone tested were 29.5, 7.4, 6.6, 4.5 and 2.8 μg/ℓ. A non-detectable level (<0.02 μg/ℓ) was also tested. One liter water samples taken from aquaria one hour after introduction of the test compound were analyzed by gas chromatography as in the method of Schimmel and Wilson (1977).

RESULTS AND DISCUSSION

Kepone was acutely toxic to lugworms at the highest concentration tested. All lugworms exposed to 29.5 μg/ℓ died while no mortalities occurred at the lower concentrations during the 144 hour period. Significant inhibition of lugworm feeding activity in both magnitude and rate was observed at all detectable levels of Kepone tested ($F \geq 91$, d.f. 2, 10; $\infty = 0.01$). Comparisons of substrate reworking rates between exposed and control lugworms for selected tests are shown in Figure 4.

Lugworms were sensitive to Kepone at sub-lethal levels as low as 2.8 μg/ℓ. During the first 48 hours of exposure to concentrations ranging from 7.4 to 2.8 μg/ℓ exposed and control lugworms displayed similar rates of feeding funnel formation. However, between 60 and 144 hours a significant reduction in the amount of surface area disturbed by exposed lugworms was observed. This latent effect suggests that lugworms may gradually accumulate Kepone until a threshold level is reached which then interrupts the "Normal Cyclical Pattern."

The relative toxicity of Kepone to several estuarine species has been determined (Schimmel and Wilson 1977, Nimmo *et al*. 1977). The species examined, and their 96-hour LC50 values were: grass shrimp (*Palaemonetes pugio*), 121 μg/ℓ; blue crab (*Callinectes sapidus*), 210 μg/ℓ; mysid (*Mysidopsis bahia*), 10.1 μg/ℓ; sheepshead minnow (*Cyprinodon variegatus*), 69.5 μg/ℓ; and the spot (*Leiostomas xanthurus*), 6.6 μg/ℓ. Although the 96-hour LC50 value for the lugworm was not determined, complete mortality was observed within 48 hours at 29.5 μg/ℓ. Based on these findings it is apparent that the lugworm is sensitive to Kepone when compared with other estuarine species.

General Conclusions

I have presented a bioassay system for quantifying the effects of a pollutant on a marine infaunal polychaete. The toxicity tests conducted with Kepone serve to illustrate applicability of the bioassay technique. At sublethal concentrations (7.4 to 2.8 μg/ℓ) the Normal Cyclical Pattern of the lugworm was interrupted, resulting in a decrease in substrate reworking activity. Although the consequences of reduced lugworm activity are speculative at this time, it is possible that a suspension of substrate reworking by the lugworm and other infaunal organisms with similar deposit feeding habits could reduce the exchange of pollutants between the sediment and water column thereby prolonging the residence time of a pollutant in the water. Long term effects of reduced lugworm feeding activity could eventually result in the depaupuration of lugworm populations. Such an event would affect the overall transport of nutrients and pollutants through the benthic system as well as alter food chains of which the lugworm forms a part.

Whatever the environmental significance of this deviation from normal activity caused by pollutant stress, it is clear that this technique can demonstrate a behavioral effect on an important infaunal organism. Such a test which demonstrates biological effects of low levels of pollutants on an important ecological process such as sediment reworking will be of value in determining the potential impact of a contaminant on the marine environment.

ACKNOWLEDGMENTS

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Figure 1. Feeding funnels produced by one lugworm at 12-hour intervals.

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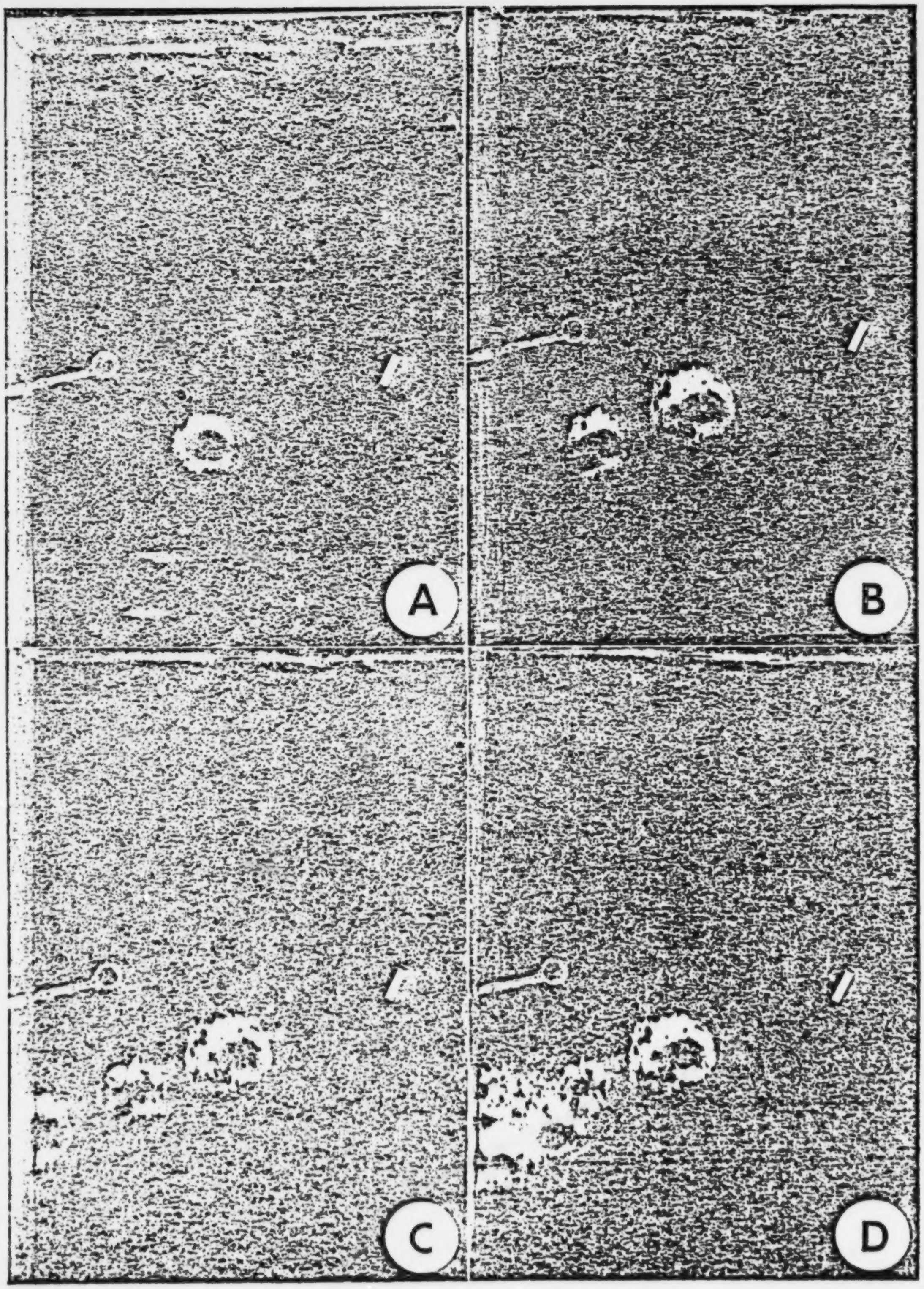


Figure 2. Photo-Bioassay System (A-24-hour timer, B-35 mm camera with automatic advance, D, E aquaria with 25 cm of sand and 75 ℓ of seawater).

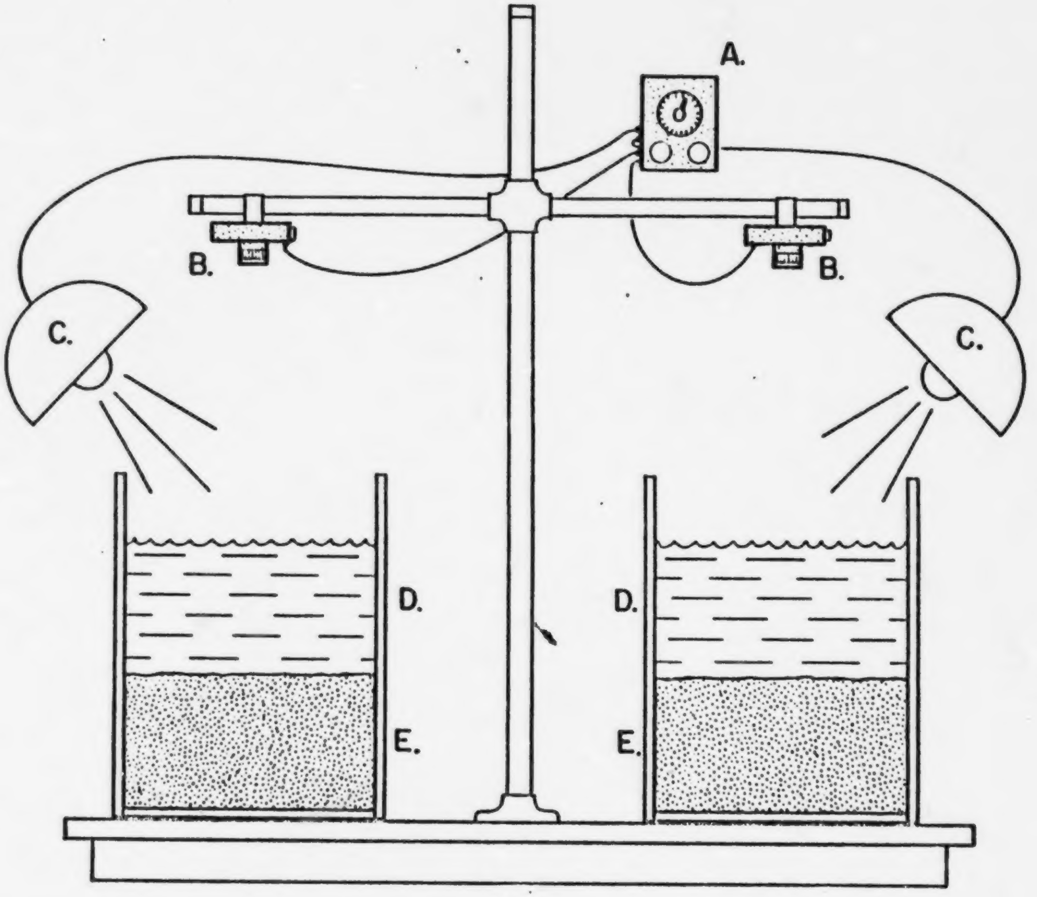

A.
B.
B.
C.
C.
D.
D.
E.
E.

Figure 3. Comparison of the rates of sediment turned under by groups of lugworms of similar size. A different group of lugworms was used for the six replicate tests.

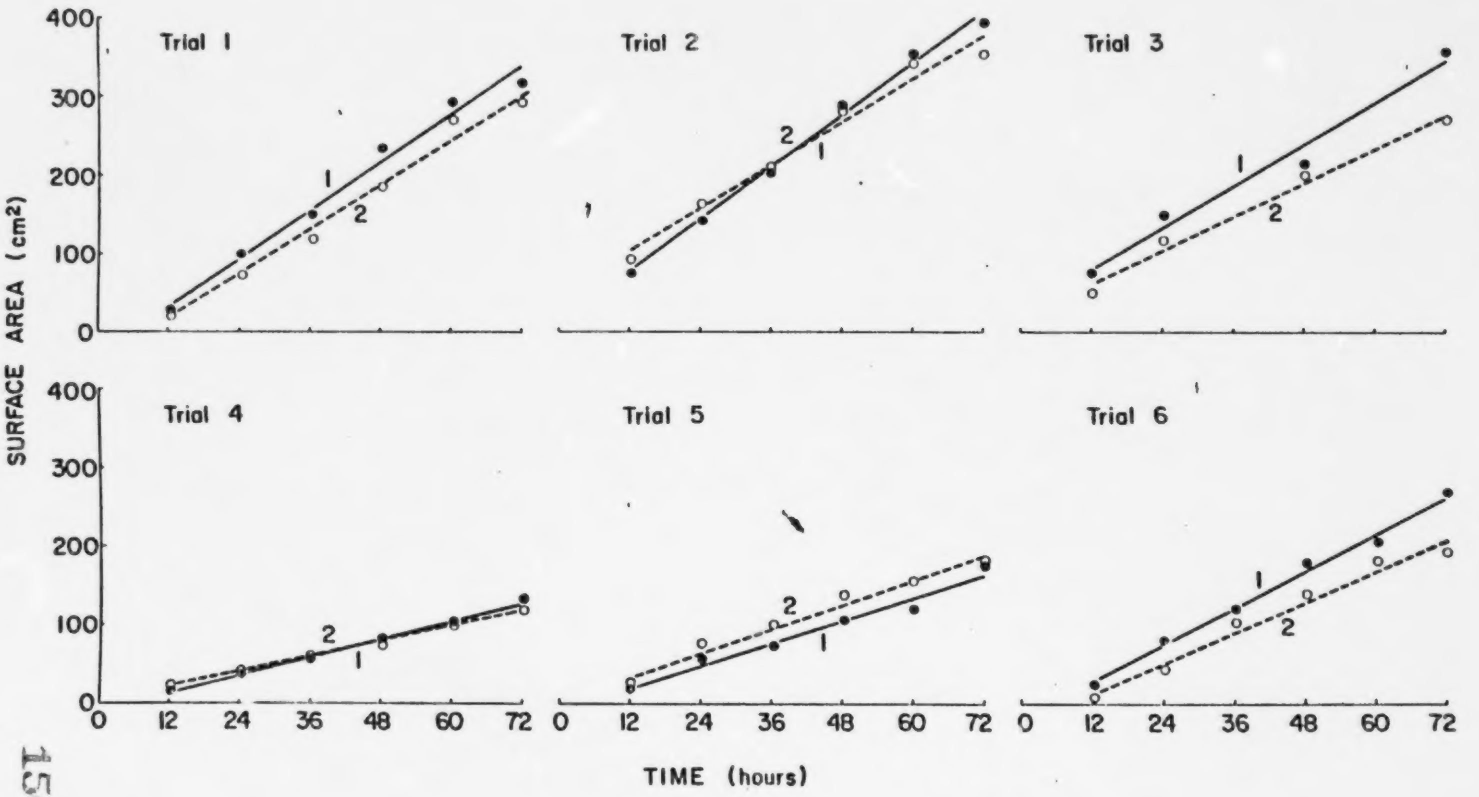
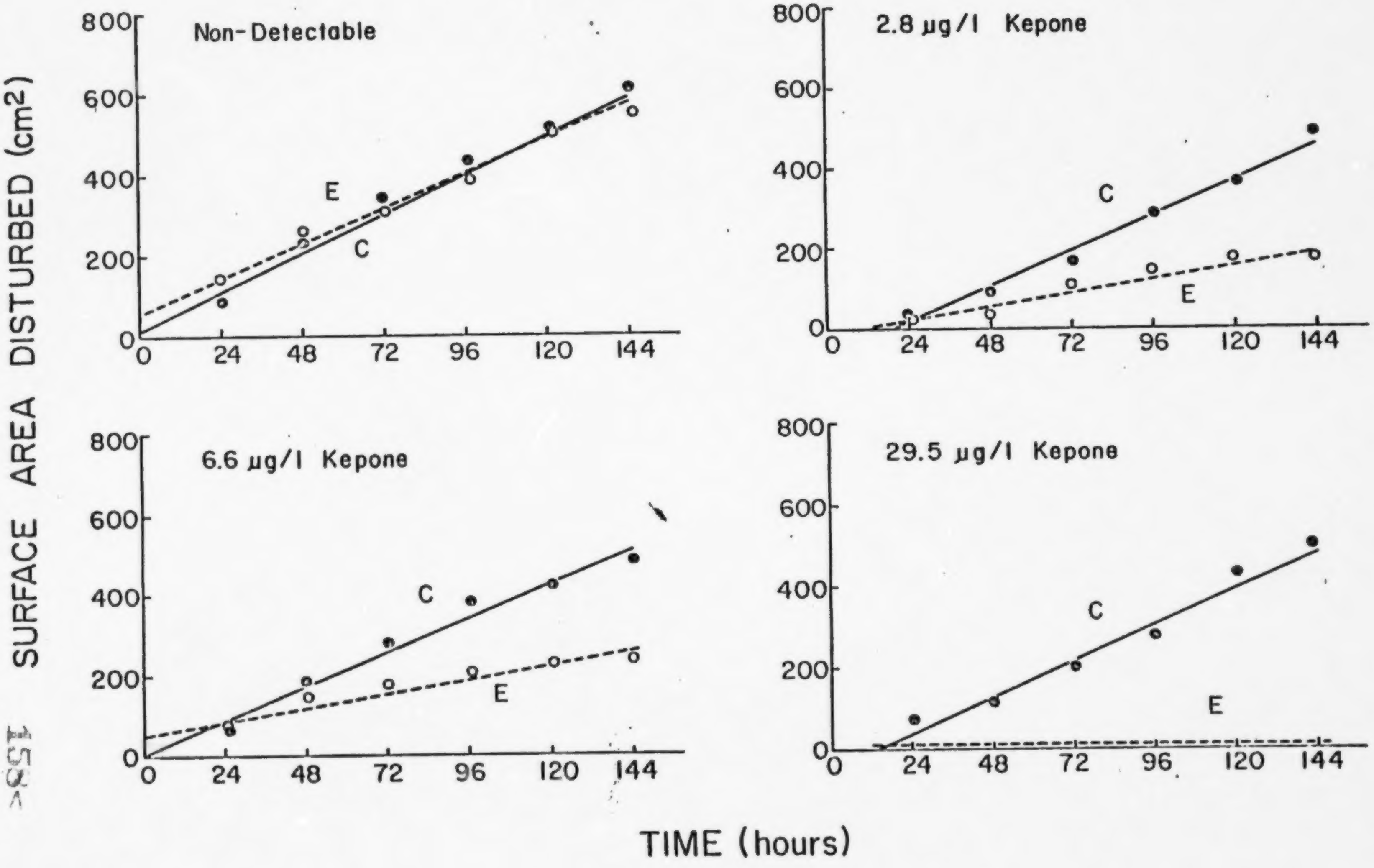

Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
1
2
SURFACE AREA (cm²)
0
100
200
300
400
TIME (hours)
0
12
24
36
48
60
72

Figure 4. Comparison of the rates of sediment turned under by lugworms. C: control; E: experimental group exposed to Kepone. Each group consisted of six lugworms.


SURFACE AREA DISTURBED (cm2)
TIME (hours)
Non-Detectable
2.8 µg/l Kepone
6.6 µg/l Kepone
29.5 µg/l Kepone
E
C
0
200
400
600
800
0
24
48
72
96
120
144

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Kepone^R: Toxicity and Bioaccumulation in Blue Crabs

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Estuaries (In Press)

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ABSTRACT

Two long-term studies were conducted to determine toxicity, uptake and depuration of Kepone in blue crabs (Callinectes sapidus). In the first, Kepone was administered to crabs in seawater (0.03 or 0.3 μg Kepone/ℓ) or food (eastern oyster, Crassostrea virginica, containing 0.25 μg/g Kepone). Uptake of Kepone in 28 days was primarily through the contaminated oysters. When these crabs were held in Kepone-free seawater and fed Kepone-free oysters for 28 days, no loss of the insecticide was evident. There were adverse effects on molting and survival in crabs fed oysters that contained 0.25 μg/g Kepone.

A second study was conducted to determine: (1) the depuration of Kepone over a 90-day period in blue crabs fed oysters from the James River, Virginia (containing 0.15 μg/g Kepone); and (2) the effects of Kepone on molting and survival of blue crabs fed James River oysters or laboratory-contaminated oysters that contained 0.15 or 1.9 μg/g Kepone. Crabs fed Kepone-contaminated oysters followed by a diet of Kepone-free oysters for 90 days had detectable concentrations

of the insecticide in tissues. Also, blue crabs that ate oysters containing Kepone in concentrations similar to those found in oysters from the James River, died or molted less frequently than crabs fed Kepone-free oyster meats.

INTRODUCTION

Contamination of Virginia's James River estuary by Kepone in the late 1960's and early 1970's, and its transport into Chesapeake Bay, raised questions about the chemical's persistence in and effects on the area's estuarine biota. Kepone residues in James River biota, in particular blue crabs (*Callinectes sapidus Rathbun*), bluefish (*Pomatomus saltatrix Linnaeus*), and eastern oysters (*Crassostrea virginica* Gmelin), were found by the U.S. Food and Drug Administration (FDA) to be sufficiently high to limit the commercial harvesting of these and other species captured in the estuary.

Kepone residues in James River blue crabs coincided with a decrease in commercial crab landings. Residues in males was 0.81 μg/g; in females, 0.19 μg/g (Bender et al., 1977). Total blue crab catch from the James River indicated a 90% decline from 1972 through 1975 (U.S. Dept. Commerce, 1968-1975).

Laboratory exposures of blue crabs in seawater to Kepone indicated relatively low acute toxicity and bioconcentration (Schimmel and Wilson, 1977). This, in contrast to James River residue data, suggested that the major route of Kepone entry in crabs may be through contaminated food.

In this paper, we report the results of two long-term studies to determine: (1) uptake and depuration rates of Kepone in blue crabs exposed to the insecticide in seawater and food (eastern oysters); and (2) effects of Kepone in food on molting and survival of blue crabs.

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METHODS AND MATERIALS

Test animals

Blue crabs for both studies were collected from Santa Rosa Sound, Florida; oysters, used as food for blue crabs, were collected either from Santa Rosa Sound (local oysters) or from Wreck Shoals in the James River estuary, Virginia. Eastern oysters were appropriate food in these bioaccumulation studies because: (1) oysters bioconcentrate Kepone under laboratory conditions (Bahner et al., 1977); (2) oysters contaminated with Kepone have been reported from the James River (Bender et al., 1977); and (3) oysters are a natural food of blue crabs (Anonymous, 1941; Loosanoff, 1948; Lunz, 1947; Menzel and Hopkins, 1956; and Menzel and Nichy, 1958). Approximately 95% of the crabs collected were _Callinectes sapidus_; the remaining, _C. similis_ (Williams). No effort was made to separate the latter species because of the limited numbers of animals available and the need to avoid excessive handling. Locally collected oysters (Santa Rosa Sound) and blue crabs contained no Kepone detectable by gas chromatographic analysis. Blue crabs were acclimated to laboratory conditions for at least 14 days prior to testing.

Study No. 1

Approximately 450 juvenile blue crabs (19-49 mm carapace width, $\bar{x}$ = 36.7 mm) were collected from Santa Rosa Sound, in July 1976. During acclimation, crabs were fed meats of eastern oysters from Santa Rosa Sound ad libitum.

A 56-day bioconcentration (uptake directly from the exposure water) and bioaccumulation (uptake primarily from ingested food) study was conducted, using the diluter described by Schimmel et al. (1977). Seawater was pumped from Santa Rosa Sound through a sand filter and into a reservoir in the laboratory. It was then heated to a mean temperature of 22.1°C (range, 20.0 to 23.5°C); salinity was allowed to vary with sound conditions (range 20.0 to 31.0 ‰; $\bar{x}$ = 25.5 ‰). From the reservoir, seawater was pumped to the diluter mixing box by Lambda^R pumps. The diluter provided two Kepone concentrations in seawter (0.03 µg/ℓ and 0.3 µg/ℓ, nominal

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concentrations), each concentration duplicated, as well as seawater free of Kepone, also duplicated. Six exposure aquaria measured 46 cm x 71 cm x 26 cm (volume = 86ℓ), and the diluter delivered one liter to each aquarium each cycle. There was an average of 300 cycles each day (10 turnover volumes). Fifty-six blue crabs were placed simply in individual compartments (6.5 cm x 4.7 cm x 10.0 cm) in each aquarium. At least 12 holes (6 mm diameter) were drilled in each compartment to provide adeuqate circulation.

The 56-day study was divided into a 28-day uptake period and a 28-day depuration period. In the uptake period, Kepone was administered to the crabs; (1) in the water delivered to the experimental aquaria; and (2) in contaminated, eastern oysters as a food source. Adult oysters were contaminated by exposing them to 0.1 µg/ℓ Kepone for 42 hours; afterwards oyster meats were removed from their shells, pooled, and analyzed quantitatively for Kepone content. Approximately 0.5 g of the oysters, contaminated with 0.25 µg/g Kepone, were fed to the crabs twice each week in a control aquarium, and to crabs exposed either to the 0.03 µg/ℓ or 0.3 µg/ℓ. Approximately 0.5 g of uncontaminated, Santa Rosa Sound oysters (< 0.02 µg/g Kepone) were fed to crabs in the remaining control and experimental aquaria (Table 1). Two crabs were sampled from each aquarium during the uptake portion of the test at 1 day, 2 days, and, after the second day, twice each week to the end of the 28-day exposure period. Identical sampling intervals were used for the 28-day depuration portion of the study. When sampled, each crab was dissected into muscle and remaining tissues including exoskeleton. Muscle tissue was obtained from the thoracic region and contained small amounts of chitin. Remaining tissues consisted of whole crab not included in the muscle tissue sample. Molting and mortality of crabs were monitored daily throughout the study.

Study No. 2

Approximately 580 juvenile blue crabs (9 to 42 mm carapace width, $\bar{x}$ = 28 mm) and approximately 1000 adult eastern oysters were collected from Santa Rosa Sound in February and March, 1977.

Uncontaminated local oysters, the control food, were removed from their shells, cut into 0.5 g portions, and frozen.

Two hundred local, uncontaminated oysters were placed in an aquarium and exposed to 1.0 μg/ℓ Kepone for 20 hours to obtain an average of 1.9 μg/g Kepone in their tissues. Another 200 oysters were exposed to 0.1 μg/ℓ to obtain an average of 0.15 μg/g Kepone. Oysters from each aquarium were then cut into 0.5 g pieces and frozen.

Additional eastern oysters were collected in the James River (near Wreck Shoals) in March 1977 by the staff of the Virginia Institute of Marine Sciences and shipped by air freight to the Gulf Breeze Environmental Research Laboratory. Gas chromatographic analysis of pooled oysters showed a Kepone concentration of 0.15 µg/g wet weight. These oysters were also cut into 0.5 g pieces and frozen.

Study No. 2 with blue crabs was conducted to determine: (1) the effects of Kepone on molting and survival; and (2) the rate of depuration of Kepone beyond the 28-day period tested in Study No. 1. The same exposure apparatus used in Study No. 1 was used except that no Kepone was administered in seawater. Six aquaria were used, each containing 68 blue crabs (held in individual compartments as in the previous study). Crabs in aquaria Nos. 1 and 4 were used to determine uptake and depuration of Kepone administered through their food; those in aquaria Nos. 2, 3, 5, and 6 were observed for effects on molting or survival (Table 2). Crabs in aquarium No. 1 were fed 0.5 g of Kepone-free oysters twice each week. Crabs were sampled twice each week for 28 days, three per sample, for Kepone residue analyses; thereafter, crabs were sampled less frequently for an additional 90 days. Crabs were dissected into muscle and remaining tissues, as in the first study.

Chemical methods

Methods of chemical analyses of Kepone were those of Schimmel and Wilson (1977). The average recovery rate of Kepone from fortified tissue was 87%; from water, 85%. Residue concentrations were calculated on a wet-weight basis without correction for percentage recovery. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of the results.

Statistical methods

Blue crab molting data were analyzed by one-way analysis of variance followed by the Student-Newman-Keuls post hoc tests to determine significant differences ($\alpha = 0.05$); mortality data were analyzed by Student's t ($\alpha = 0.05$).

RESULTS

Study No. 1

Blue crabs fed oysters contaminated with 0.25 μg/g Kepone accumulated the insecticide readily in their muscle and remaining tissues in 28 days. However, after 28 days in a Kepone-free environment, no depuration of the compound was evident (Fig. 1). When fed 0.25 μg/g Kepone in oysters for four weeks, crabs accumulated the insecticide to an average of approximately 0.1 μg/g in both their muscle and remaining tissues. Uptake of Kepone by crabs fed contaminated oysters only (aquarium No.1) was nearly identical to that of crabs fed contaminated oysters and provided Kepone in seawater (aquaria Nos. 3 and 4), indicating that very little of the insecticide was taken in directly from water. No measurable concentrations of Kepone were detected in crabs from aquaria supplied with 0.3 μg/ℓ (0.08 μg/ℓ measured) or 0.03 μg/ℓ (0.014 μg/ℓ measured) Kepone (aquaria Nos. 5 and 2 respectively).

Blue crabs in all aquaria were monitored for molting and mortality throughout the 56-day test. Although no statistical tests could be applied to these data, we observed that crabs fed control oysters molted a total of 63 times, and 22 crabs died (Table 3). Crabs fed Kepone-contaminated oysters for 28 days, and control oysters for 28 additional days, had fewer molts (n=48) and more deaths (n=49) than crabs fed only control oysters. The data indicated that Kepone in crab food may reduce their ability to molt or survive. Therefore, we conducted Study No. 2 to investigate this possibility and to determine the depuration rate of Kepone in crabs over a longer period than in the previous study.

Study No. 2

Blue crabs in aquarium No. 4 that were fed James River oysters (containing 0.15 μg/g Kepone) accumulated the insecticide in muscle and whole-body tissues (muscle and remaining tissues) to a maximum of 0.069 μg/g in 28 days (Fig. 2). After a Kepone-free diet for 90 days, crabs showed some loss of the insecticide. However, 0.025 μg/g Kepone was detected in remaining tissues, and approximately 0.014 μg/g remained in muscle tissues. Some of this apparent loss of Kepone must be attributed to growth of the blue crabs. No length or weight measurements were made at the end of the 28-day exposure period, and consequently the exact growth could not be determined.

Molting by crabs fed James River oysters (aquarium No. 3, Kepone concentration = 0.15 μg/g), oysters containing 0.15 μg/g Kepone (aquarium No. 5), or 1.9 μg/g Kepone (aquarium No. 6) was significantly reduced ($\alpha = 0.05$) compared with those fed control oysters (aquarium No. 1, Fig. 3). The average molts per crab in 56 days were: control, 1.4; 1.9 μg/g Kepone, 0.56; 0.15 μg/g Kepone, 0.97; and James River oysters, 0.48.

Percent mortality in crabs fed oysters containing 1.9 μg/g Kepone and those fed James River oysters was significantly greater ($\alpha = 0.05$) than those fed control oysters (Fig. 4). Blue crabs which ate oysters containing 1.9 μg/g Kepone exhibited extreme excitation, especially during feeding. In the advanced stages of Kepone poisoning, the crabs were generally lethargic for several days before death. No crabs in any other aquaria displayed these signs. Percent mortality of crabs fed oysters containing 0.15 μg/g Kepone was not significantly different from that of crabs fed control oysters.

DISCUSSION

The uptake and depuration of Kepone by blue crabs in both studies help explain why relatively high Kepone residues are found in crabs from the James River, Virginia. No discernible depuration of Kepone in crab muscle or remaining tissues was evident in 28 days but some loss of Kepone was apparent beyond 28 days. However, detectable Kepone was present in tissues of crabs held 90 days in a Kepone-free environment. In the James River, Bender et al. (1977) reported that average Kepone concentrations in estuarine vertebrates and invertebrates ranged from 0.09 to 2.0 μg/g. Many, if not all, of the species listed are included in the diet of the blue crab. Therefore, it is reasonable to conclude from our laboratory studies and James River field data that Kepone residues will persist in blue crab tissues as long as detectable concentrations remain in the food.

Blue crabs fed oysters containing 1.9 μg/g Kepone exhibited signs of poisoning similar to crabs fed fish contaminated with 1.0 μg/g of mirex (Lowe et al., 1971). Mirex is an organochlorine insecticide chemically similar to Kepone. Lowe et al. (1971) also reported that juvenile blue crabs exposed to 100 μg/ℓ mirex in flowing seawater for 96 hours showed no signs of poisoning. Schimmel and

Wilson (1977) exposed blue crabs to Kepone for 96 hours at concentrations as high as 210 μg/ℓ and reported no significant mortality.

Blue crabs fed oysters from the James River (containing 0.15 μg/g Kepone) died in significantly greater numbers and molted fewer times than those fed uncontaminated oysters; crabs fed oysters containing 0.15 μg/g Kepone (by laboratory exposure) moulted fewer times but did not die in significantly different numbers than those fed uncontaminted oysters. Reasons for the difference in mortality are unclear. However, it should be noted that a 0.15 μg/g Kepone residue appears to be the threshold level for effect: blue crabs fed local oysters contaminated with 1.9 μg/g Kepone suffered 80% mortality, and those fed local oysters contaminated with 0.25 μg/g Kepone died in greater numbers than those fed control oysters. It is possible that the difference in mortality in the crabs fed James River oysters (0.15 μg/g) and those fed local, contaminated oysters (0.15 μg/g) may be due to a toxicant not detectable by our methods of chemical analysis or that our methods of exposure and duration were different than those of oysters from the James River. To investigate the possibility of some metal in the oysters, samples of local and James River oysters were sent to the EPA Environmental

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Research Laboratory, Athens, Georgia, for analysis. Results of these analyses (multielement analysis by plasma emission and spark source mass specrometry) indicated that oysters from the James River contained approximately 5 x more aluminum (70 μg/g), 10 x more copper (29 μg/g), and 9 x more zinc (415 μg/g) than local oysters. Concentrations of other metals in oysters from both areas were similar. We do not know the effects of these metals and Kepone on the survival of blue crabs.

Annual crab fishery statistics (U.S. Dept. Comm., 1968-197~~6~~5) from the James River show that from 1968 to 1972 the commercial catch ranged from 691,000 kg to 1,211,000 kg ($\overline{x}$ = 899,000 kg), but from 1972 through 1975 the catch decreased more than 90% to 14,700 kg. (A moratorium on harvesting of blue crabs in the James River was in effect in 1976). The blue crab catch in the adjacent Rappahannock River estuary from 1968 through 1975 did not show a significant decline. From these catch data it appears that some pertubation is causing a particularly adverse affect on blue crab abundance in the James River estuary.

If the James River estuary had been affected by only one pollutant, it would have been comparatively easy to determine a cause and effect relationship with that pollutant and the decrease in the crab fishery. As in nearly all instances in the natural environment, this is not the case in the heavily industrialized James River area. However, based on our laboratory data, we believe that Kepone may be a factor in the decline of the James River crab fishery.

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________________AND A.J. WILSON, JR. 1977. Acute toxicity of Kepone to four estuarine animals. Chesapeake Sci. 18:224-227.

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Table 1. Concentrations of Kepone used in Study No. 1 for 28-day exposure of blue crabs (*Callinectes spp.*) to the insecticide in seawater or in food (eastern oyster, *Crassostrea virginica*).

AQUARIUM	KEPONE[1]	
	WATER (μg/ℓ)	FOOD (μg/ℓ)
1	ND[2]	0.25
2	0.03	ND
3	0.03	0.25
4	0.3	0.25
5	0.3	ND
6	ND	ND

---

[1] Uptake period was followed by a 28-day depuration period in which no Kepone was administered in food or in seawater.

[2] ND - non-detectable: < 0.02 μg/ℓ in water, <0.02 μg/g in oyster tissue.

Tabel 2. Experimental design for 56-day exposure of blue crabs (*Callinectes spp*.) to the insecticide, Kepone, in eastern oysters (*Crassostrea virginica*) (Study No. 2)

AQUARIUM	SOURCE OF OYSTERS	CONCENTRATION OF KEPONE IN OYSTERS (µg/g)	OBSERVATION
1	Santa Rosa Sound, Florida	ND[1]	Molting and Survival
2	Santa Rosa Sound, Florida	ND	Uptake/Depuration
3	James River, Virginia	0.15	Molting and Survival
4	James River, Virginia	0.15	Uptake/Depuration
5	Santa Rosa Sound, Florida	0.15	Molting and Survival
6	Santa Rosa Sound, Florida	1.9	Molting and Survival

[1] ND = non-detectable; <0.02 µg/g in tissues.

Tabel 3. Total molts and mortality of blue crabs (Callinectes spp.) in Kepone Study No. 1. Fifty-six crabs were placed in each aquarium at the beginning of the test.

Aquarium No.	Test Conditions	Molts	Mortality
1	Control water[1]; 0.25 μg/g Kepone in oyster	18	15
2	0.03 μg/ℓ Kepone in water; Control oysters[1]	20	6
3	0.03 μg/g Kepone in water; 0.25 μg/g Kepone in oysters	12	20
4	0.3 μg/ℓ Kepone in water; 0.25 μg/g Kepone in oysters	18	14
5	0.3 μg/ℓ Kepone in water; Control oysters	22	9
6	Control water; Control oysters	21	7

[1] Control water, <0.02 μg/ℓ Kepone; Control oysters, <0.02 μg/g Kepone.

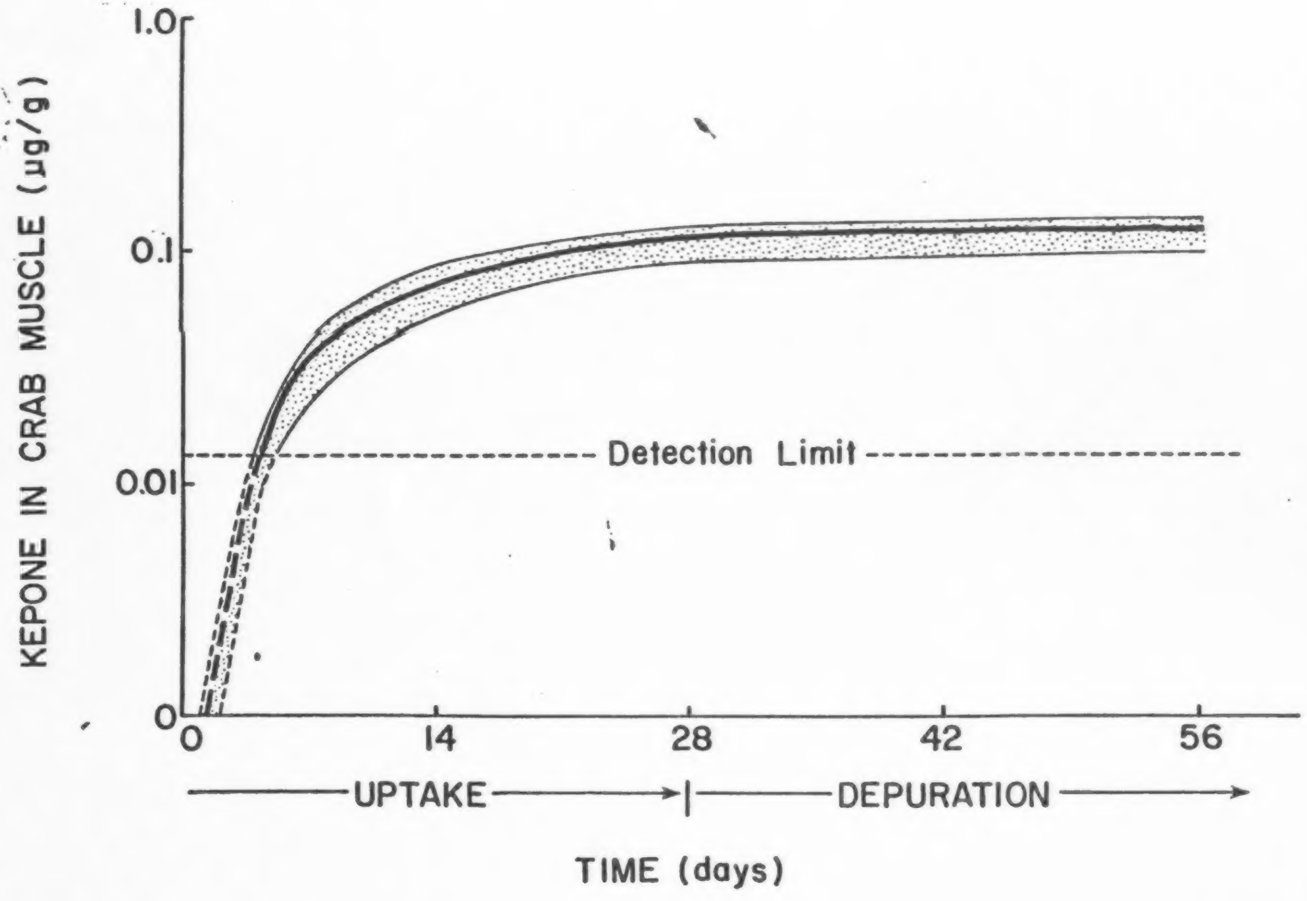
KEPONE IN CRAB MUSCLE (μg/g)
1.0
0.1
0.01
0
Detection Limit
0
14
28
42
56
UPTAKE
DEPURATION
TIME (days)

Figure 2. The 28-day accumulation of Kepone by blue crabs (*Callinectes spp.*) fed James River oysters that contained 0.15 μg/g Kepone. In the depuration period, crabs were fed Kepone-free (<0.02 μg/g) oysters. Kepone residue for muscle on day 118 is estimated to be 0.014 μg/g.

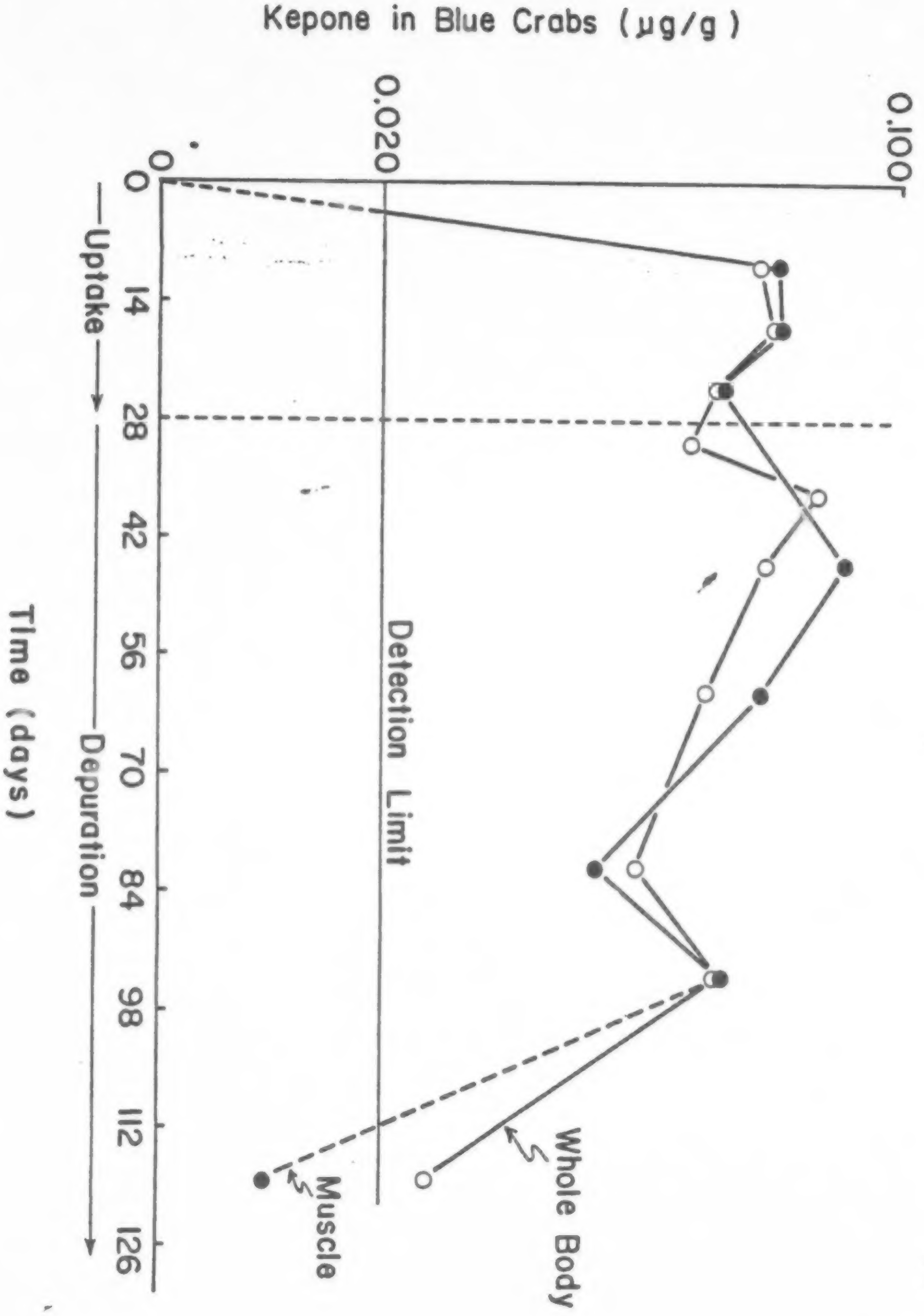
Kepone in Blue Crabs (µg/g)
0.100
0.020
0
0
14
28
42
56
70
84
98
112
126
—Uptake—
—Depuration—
Time (days)
Detection Limit
Whole Body
Muscle

Figure 1. Bioaccumulation of Kepone in muscle tissues of blue crabs (Callinectes spp.) fed oysters contaminated with 0.25 μg/g of the insecticide for 28 days followed by a 28-day period for depuration. The uptake curve and the 95% confidence interval represented are a composite of three homogeneous curves representing uptake in crabs fed: (1) 0.25 μg/g Kepone in oysters and control seawater, (2) 0.25 μg/g Kepone in oysters and 0.03 μg/ℓ in seawater, and (3) 0.25 μg/g in oysters and 0.3 μg/ℓ in seawater.

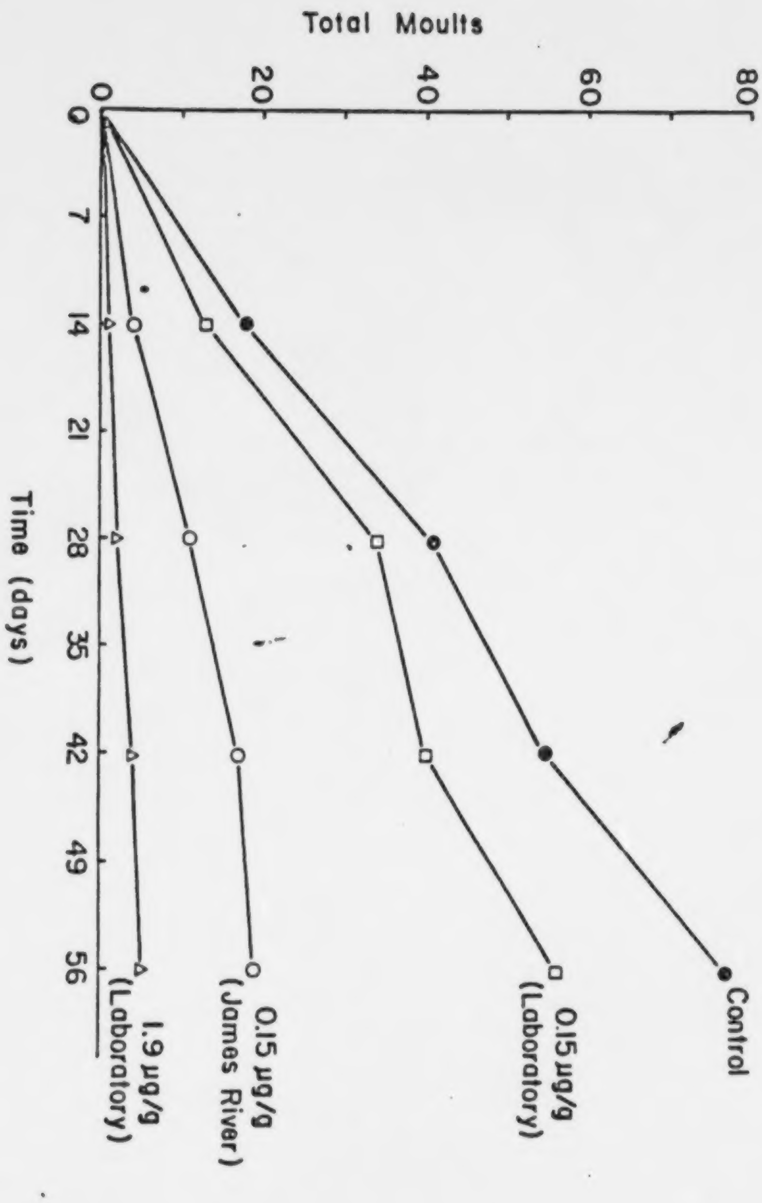
Total Moults
0
20
40
60
80
0
7
14
21
28
35
42
49
56
Time (days)
Control
0.15 µg/g (Laboratory)
0.15 µg/g (James River)
1.9 µg/g (Laboratory)

Figure 3. Molting rate of blue crabs (*Callinectes spp*.) fed uncontaminated (<0.02 µg/g Kepone) or Kepone contaminated eastern oysters (*Crassostrea virginica*) for 56 days on a flow-through bioassay. Molting rates are for crabs that survived the entire study in each test aquarium. The test conditions and numbers of surviving crabs are: control, 57 crabs; 0.15 µg/g Kepone, 58 crabs; James River, 40 crabs; and 1.9 µg/g Kepone, 9 crabs.

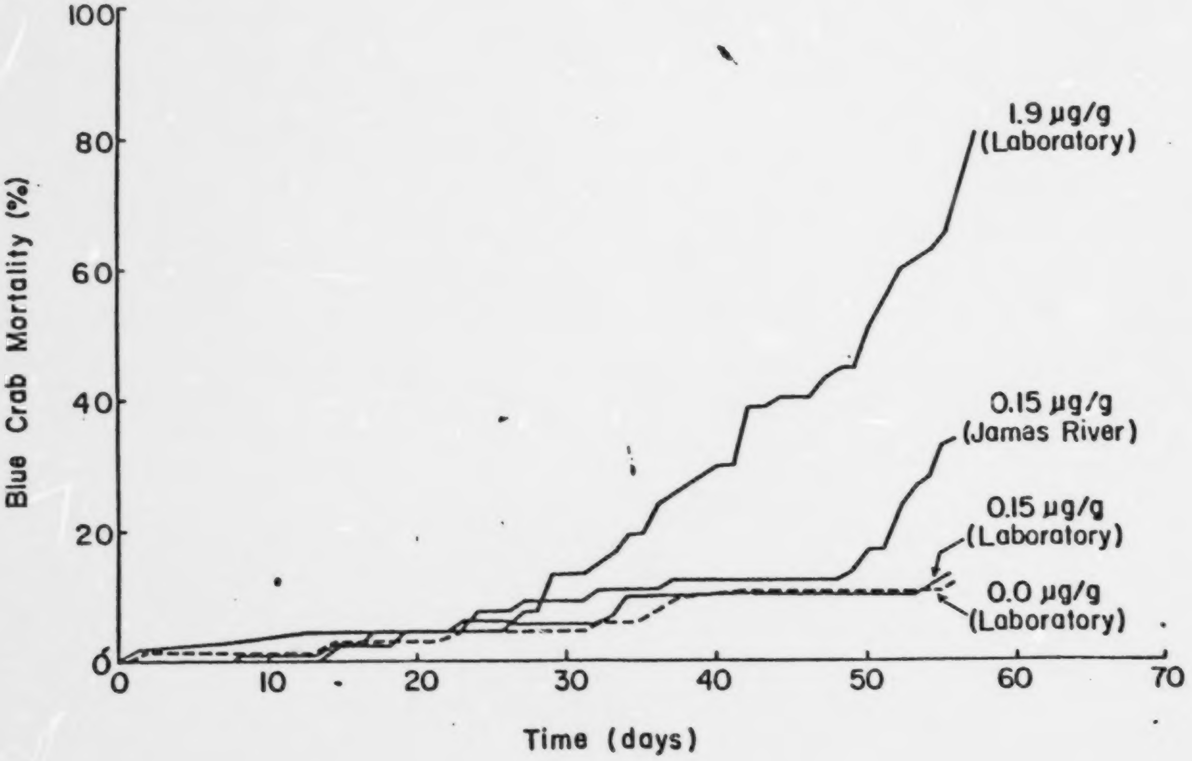
Blue Crab Mortality (%)
0
20
40
60
80
100
0
10
20
30
40
50
60
70
Time (days)
1.9 µg/g (Laboratory)
0.15 µg/g (Jamas River)
0.15 µg/g (Laboratory)
0.0 µg/g (Laboratory)

Figure 4. Mortality of blue crabs (*Callinectes spp.*) fed uncontaminated (<0.02 µg/g Kepone) or Kepone contaminated eastern oysters (*Crassostrea virginica*) for 56 days in a flow-through bioassay.

John D. Costlow

QUARTERLY PROGRAM REPORT

June - September 1977
Environmental Protection Agency
Grant #R803838-02-0

Effects of Insect Growth Regulators and Juvenile Hormone Mimics on Crustacean Development

Effect of Juvenile Hormone Mimics and Insect Growth Regulators on Larval Development of Brachyura

Four different experiments were undertaken as continuations of earlier work on the effects of Dimilin^{R} (TH-6040) on larval development of several marine Brachyura. The first experiment was to determine the rate of deterioration of Dimilin in seawater through bioassay and, subsequently, chemical analysis. The second experiment was designed to determine the degree of sensitivity of individual larval stages to Dimilin. The third experiment was designed to examine differential sensitivity to Dimilin during particular times of the intermolt period of the larvae. The fourth experiment involved preparation of larvae exposed to Dimilin during specific stages of development for electron microscopy studies of cuticle.

As indicated in the previous quarterly report (March-May, 1977) volumes of 10 ppb Dimilin were prepared in 20 ppt seawater, stored in natural daylight at room temperatures, and used for a period of approximately 70 days as the culture solution for successive hatches of larvae of Rhithropanopeus harrisii. As indicated in Table I, there was a high mortality of larvae during the first stage of development in all series cultured up to 69 days following preparation of the Dimilin solution. All larvae succumbed, in some cases after successfully completing zoeal development, by varying times, ranging from eight days following hatching to 17 days. When the solution was 29 days old, 25% survived to the second zoeal stage but these larvae died before or during molting to the third zoeal stage. Nearly all larvae appeared normal in morphological and behavioral respects until they began to molt to the second zoeal stage. At that time, the majority died during molting, frequently without being able to remove the old exoskeleton. Those that did manage to complete the molt were abnormal with deformed swimming setae and were in capable of normal swimming. There were other morphological defects, including rostral and antennal spines. In all controls, survival ranged from 89 to 99 percent with an extremely small percentage (1-4) of abnormalities throughout all of the zoeal stages.

Earlier experiments in this laboratory have shown that 10 ppb Dimilin is lethal to R. harrisii larvae when they are exposed at the time of hatching and that only a few larvae will survive beyond the first zoeal stage. As shown in Table II, 10 ppb Dimilin is lethal to other stages as well. One hundred percent mortality occurred during molting when the larvae were exposed to 10 ppb Dimilin in the second stage, the third stage, and the fourth zoeal stages, whereas 97-99% survived in the control series without Dimilin. The megalopa, however, did not appear to be as sensitive to this compound as the zoeal larvae. When megalopa are exposed to Dimilin, 44% did survive to the crab stage; within the control series, 91% survived.

First zoeal larvae of Rhithropanopeus harrisii larvae were exposed to 10 ppb Dimilin at various times within one intermolt period. At 25°C, molting to the second stage begins on day 4. Individual larvae were exposed to 10 ppb Dimilin on day 1, day 2, or day 3, on days 1 and 2, on days 2 and 3, and on days 1 through 3. Results, as shown in Table III, indicate that the larvae were more sensitive to the compound late in the intermolt period than at the beginning of the intermolt period. The variations in survival of replicates are probably due to the time exposure relative to the time of hatching. Exposure to Dimilin was initiated in the mornings whereas the larvae could have hatched at any time between the previous evening and the early morning when exposure was initiated.

A few zoeal larvae exposed to 10 ppb Dimilin, as well as larvae from the control series, were fixed for electron microscopy studies in glutaraldehyde and OsO_4. Some exposed first stage zoeae were fixed during molting to the second zoeal stage. Sections of the dorsal spine have recently been prepared and preliminary studies under the electron microscope indicate that the cuticle is considerably altered in those larvae that have been exposed to Dimilin when compared with larvae within the control series.

Effects of Juvenile Hormone Mimics and Insect Growth Regulators on Behavior of Brachyuran Larvae

The manuscript entitled "Sublethal Effects of Insect Growth Regulators upon Crab Larval Behavior" was accepted for publication by Water, Air and Soil Pollution. This work clearly indicated that sublethal concentrations of Dimilin and hydroprene alter phototaxis and swimming by Rhithropanopeus harrisii larvae. Thus work this summer has focused on considering the functional significance of responses to light and other environmental variables.

The main study determined whether the change in phototactic pattern upon light and dark adaptation could serve as the basis for a diurnal vertical migration pattern. The accumulated evidence indicated that this probably does not occur. This work was presented at the 12th European Marine Biological Symposium (September, 1977).

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A second area of intensive investigation was a field study of R. harrisii larval distributions in relation to environmental factors. At a fixed station in the Newport River Estuary hourly vertical samples were taken of larvae, light intensity, temperature, salinity and currents. This was carried out twice with the first session lasting four days. The second was terminated after two and a half days due to a mechanical breakdown. The collected samples are now being analyzed. It is anticipated that this information will indicate which environmental factors are important in establishing crustacean larval distributions within an estuary.

Effects of Kepone on Development of Callinectes sapidus and Rhithropanopeus harrisii

Preparation for Experiments

Disposal of Kepone Solutions. In response to a telephone conversation to CHEM-DYNE CORPORATION, Bruce A. Whitten, President, sent me a letter July 15, 1977 stating that their disposal fee in 55-gallon drum quantities would be $50 per drum F.O.B., Hamilton, Ohio. He suggested that we pour our Kepone contaminated brine from finger bowls into one-gallon plastic jugs and cap the jugs. The filled jugs could then be packed into 55-gallon open-top steel drums. The spaces between and above jugs could be filled with VERMICULITE. The drums should be labeled "Waste Brine Solution" and shipped to CHEM-DYNE CORP., Hamilton, Ohio. Our drums will be consolidated with others to form a full drum truckload which will be disposed of in full compliance with all Federal, State, and local environmental regulations by secured chemical landfill at the same site used by the U.S. Government for radioactive wastes.

On July 18, 1977 Mr. William L. Blake, Safety Coordinator, Duke University, and Dr. Conrad M. Knight, Radiological Officer of Duke University-Duke University Medical Center were informed by phone of our plans to dispose of Kepone. On July 20 the same information was put in a letter and sent to Mr. Blake. On July 18, 1977, I also phoned the North Carolina Department of Agriculture, Pest Control Division, and talked to Larry D. Perry, Pesticide Specialist, EPA coordinator, about our experiments and methods of disposing of brine contaminated Kepone. On July 19, 1977 Mr. Larry D. Perry wrote me that his office approved of our suggested method of disposing of waste Kepone. Mr. Blake and Dr. Knight also approved of CHEM-DYNE CORPORATION's plan. Mr. Larry D. Perry suggested as an alternative that we send our wastes to Rollins Environmental Services, P. O. Box 221, Bridgeport, NJ 08014.

Supplies. Orders for supplies of essential glassware, plastics, pipettors, chemicals and a utility cart were placed and goods received. Chem Services were slow in filling our order for Kepone and charged $120.00 for 10 grams rather than $34.58 as in order placed by EPA, Gulf Breeze on December 15, 1975.

Open-top 55 gallon steel drums and vermiculite were furnished by Dr. Conrad M. Knight, Radiological Safety Officer of Duke University-Duke University Medical Center and one-gallon plastic jugs were contributed by DUML personnel.

Preparation of Kepone Stock Solutions. Dr. Adam Zsolnay, chemist at Duke University Marine Laboratory (DUML), prepared stock solutions by dissolving a known weight of Kepone in pesticide grade acetone and different concentrations were made up from this stock solution. The potency of some of these solutions were checked by Dr. Zsolnay after a month and a half.

Source of Ovigerous Crabs and Hatching of Eggs. Ovigerous Callinectes sapidus were collected in the Newport River near Beaufort, N.C. Four crabs with dark eggs, eyes, and visible heartbeats were selected as a source of larvae. Isolated eggs were allowed to hatch in filtered seawater with a salinity of $30^{o}/_{oo}$ in compartmented boxes and flasks on a variable speed shaker according to the method described by Costlow and Bookhout, 1960, and Bookhout and Costlow, 1975.

Ovigerous Rhithropanopeus harrisii were collected near Beaufort in August and September 1977. When they were returned to the laboratory each crab was placed in a large glass finger bowl (19.4 cm. d) containing filtered seawater with a salinity of $20^{o}/_{oo}$, the salinity to be used during rearing of larvae for experiments with Kepone. The ovigerous crabs were maintained in a constant temperature culture cabinet at 25°C and with a light regime of 12 h light and 12 h darkness until hatching occurred.

Rearing of Larvae in Range-finding Experiments. The methods of rearing larvae of R. harrisii and C. sapidus in 1 ppt acetone control and five concentrations of Kepone were essentially the same as outlined in the July 14, 1977 proposal, p. 4-7.

Direction of Work, Including Number and Kind of Experiments Conducted and Latest Results

Range finding experiments using Callinectes sapidus larvae as bioassay organisms were designed to run for 20 days. Four replicates were run with 50 freshly hatched C. sapidus larvae in each of the six media of four series (I-IV). The percent survival for each series is given in Table IV. There was differential survival with increase in concentration, but the survival in acetone control was far from satisfactory. Apparently the eggs from each of the four mother crabs were poor. In previous experiments on the effect of mirex on blue crab larvae (Bookhout and Costlow, 1975), the effects of methoxychlor on larvae of Rhithropanopeus harrisii and C. sapidus, and the effects of malathion on larvae of the same species (Bookhout and Monroe, 1977), the average survival of blue crab larvae to the crab stage over a period beyond 40 days was over 50% in acetone control. The blue crab larvae were reared earlier than those reported here. As was mentioned in the proposal, July 14, 1977, blue crab larvae in the latter part of August do not survive as well in the laboratory as those hatched in the beginning of the breeding season, the later part of April and May. Our current experiments were conducted during the first two-thirds of August. The high temperatures throughout the summer may have been partly responsible. Regardless of the cause, it is obvious that other range-finding experiments will have to be conducted in May 1978 and definitive experiments done afterwards.

Range-finding experiments using *Rhithropanopeus harrisii* larvae as bioassay organisms were initially designed to run from the time of hatching to the first crab stage. Each of the first five series of *R. harrisii* larvae used came from the eggs of a single female; the sixth series came from eggs hatched from two females.

Since running four series of *C. sapidus* larvae (1200) took an exorbitant amount of time, two series of larvae (600) were run concurrently in case of *R. harrisii*. Based on the results of the previous experiment with *C. sapidus* larvae, it was decided to use the following concentrations of Kepone to test the viability of *R. harrisii* larvae, Series I and II: 0.05, 0.10, 0.20, 0.40 and 0.80 ppb. The results are given in Table V. Since survival to the crab stage in Series I ranged from 94 to 100% in the concentrations of Kepone used compared to 92% in acetone control, it was concluded that concentrations of 0.05 ppb to 0.80 ppb Kepone were not toxic to *R. harrisii* developmental stages.

In Series III and IV concentrations of Kepone used were 0.1, 2.5, 5.0, 7.5 and 10.0 ppb. The percent survival for 11 days in these concentrations was from 96 to 100% in Series III and 92 to 100% in Series IV (Table VI). Hence, these concentrations do not affect the survival of *R. harrisii* zoeae.

In Series V and VI the range of concentrations of Kepone used were: 10.0, 20.0, 40.0, 80.0 and 160.0 ppb. Survival of zoeae for 11 days in these concentrations is given in Table VII. It appears from these experiments that survival of zoeae is not affected by 10.0 and 20.0 ppb Kepone, whereas a concentration of 40.0 ppb is sublethal and 80.0 ppb and 160.0 ppb is acutely toxic.

Predictions and Plans for the Next Reporting Period

In the next range-finding experiments the following concentrations of Kepone will be used: 20.0, 35.0, 50.0, 65.0, and 80.0 ppb. This experiment should be the last range-finding one for *R. harrisii* larvae and should furnish reliable information concerning the concentrations to use in definitive experiments. Florida crabs will be used for the next range-finding experiments, because local *R. harrisii* femlaes have reached the end of their breeding season. Arrangements will be made with a Florida supplier to ship ovigerous crabs to us in January for range-finding experiments and two other shipments for definitive experiments in February and March. Thereafter we will concentrate our efforts on range-finding and definitive experiments with blue crab larvae.

Because it will not be possible to obtain ovigerous *R. harrisii* and *C. sapidus* in the best condition during the next quarterly report we will have little to report.

Problems Encountered

1. Blue crab eggs hatched from four females on July 27, 28 and 29 proved to be less viable than those from 1972-76.

2. Our constant temperature culture cabinet broke down in October and will not be fixed until the end of October.

Current Status of Publications

Latz, M.I. and R.B. Forward. 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. Biol. Bull., 153:163-179.

Forward, R.B., Jr. and J.D. Costlow, Jr. 1977. Sublethal effects of insect growth regulators upon crab larval behavior. Water, Air and Soil Poll. In Press.

Forward, R.B., Jr. and T.W. Cronin. 1977. Crustacean larval phototaxis: possible functional significance. *In*: 12th European Marine Biological Symposium. In Press.

Publications in Preparation

Christiansen, M.E. and J.D. Costlow. 1978. Effects of Dimilin on the Development of the Mud-Crab *Rhithropanopeus harrisii*.

Christiansen, M.E. and J.D. Costlow. 1978. Bioassay studies on rate of deterioration of the compound Dimilin.

TABLE I

Age of solution when exposure was started Days	Number of Replicates Control	Number of Replicates 10 ppb	Initial Number of Larvae Control	Initial Number of Larvae 10 ppb	Survival (%) to Megalopa Control	Survival (%) to Megalopa 10 ppb	Days after hatching when all larvae were dead in 10 ppb	Mortality (%) of developmental stages in 10 ppb I	II	III	IV
0	3	3	150	150	98.0	0	12	95.3	2.7	2.0	
1	3	3	150	150	92.7	0	16	91.3	7.3	0.7	0.7
3	3	3	120	120	97.5	0	8	99.2	0.8		
5	2	2	70	70	94.3	0	13	90.0	8.6	1.4	
7	3	3	150	150	94.0	0	12	90.7	9.3		
9	2	2	100	100	93.0	0	10	99.0	1.0		
12	3	3	150	150	99.3	0	10	97.3	2.7		
16	3	3	150	150	94.7	0	9	100			
21	3	3	150	150	89.3	0	9	100			
29	3	3	150	150	93.3	0	17	74.4	24.6	0	0.7
40	3	3	150	150	*	0	12				
51	3	3	150	150	83.3	74.7	-	Days 40 - 69 Data not analyzed as yet			
59	3	3	150	150	88.7	78.0	-				
69	3	3	150	150	76.0	78.0	-				

* Controls Day 40 were terminated at Day 12 when all 10 ppb Larvae Died.

Table II

Larvae Exposed in	Number of Replicates	Control		Exposed in 10 ppb	
		Initial Number	Survival (%)	Initial Number	Survival (%)
1st stage	3	120	97.5	120	0
2nd stage	3	146	98.6	150	0
3rd stage	3	144	99.3	139	0
4th stage	4	167	98.8	170	0
Megalopa	4	109	91.7	111	44.1

TABLE III

Survival (%) to the megalopa stage when larvae were exposed at various days during the first zoeal stage.

Exposure of 10 ppb on	Control Replicate 1	Control Replicate 2	Control Replicate 3	10 ppb Dimilin Replicate 1	10 ppb Dimilin Replicate 2	10 ppb Dimilin Replicate 3
Day 1	92	86	86	48	90	88
Day 2	92	86	86	68	18	46
Day 3	92	86	86	28	0	10
Day 1 and 2	92	86		62	4	
Day 2 and 3	92	86		8	0	
Day 1 to 3	92			2		

TABLE IV

Species:	*Callinectes sapidus*	Date Hatched:	Series I 7/27/77 Series II & III 7/28/ Series IV 7/29/77
Bowl Size:	8.9 cm diam	Salinity:	$30^{o}/_{oo}$
# of Bowls:	5 per concentration	Temperature:	25°C
Larvae per bowl:	10	Light:	12-12
Total # Larvae:	50 per concentration	Diet:	*Artemia* nauplii and *Arbacia* embryos

SERIES I

Percent survival to day 20, or day when there was no survival

Acetone Control	0.05 ppb Kepone	0.5 ppb Kepone	1.0 ppb Kepone	5.0 ppb Kepone	10.0 ppb Kepone
Day 20	Day 20	Day 16	Day 17	Day 10	Day 8
2%	20%	0%	0%	0%	0%

SERIES II

Percent survival to day 20, or day when there was no survival

Acetone Control	0.05 ppb Kepone	0.5 ppb Kepone	1.0 ppb Kepone	5.0 ppb Kepone	10.0 ppb Kepone
Day 20	Day 20	Day 20	Day 15	Day 12	Day 11
4%	20%	0%	0%	0%	0%

SERIES III

Percent survival to day 20, or day when there was no survival

Acetone Control	0.05 ppb Kepone	0.5 ppb Kepone	1.0 ppb Kepone	5.0 ppb Kepone	10.0 ppb Kepone
Day 20	Day 20	Day 15	Day 14	Day 13	Day 10
18%	18%	0%	0%	0%	0%

SERIES IV

Percent survival to day 20, or day when there was no survival

Acetone Control	0.05 ppb Kepone	0.5 ppb Kepone	1.0 ppb Kepone	5.0 ppb Kepone	10.0 ppb Kepone
Day 20	Day 20	Day 17	Day 17	Day 11	Day 9
12%	14%	0%	0%	0%	0%

TABLE V

Species: Rhithropanopeus harrisii
Bowl Size: 8.9 cm diam
# of Bowls: 5 per concentration
Larvae per bowl: 10
Total # Larvae: 50 per concentration

Date Hatched: 8/18/77
Salinity: 20°/oo
Temperature: 25°C
Light 12-12
Diet: Artemia nauplii

SERIES I

	Acetone Control	0.05 ppb Kepone	0.10 ppb Kepone	0.20 ppb Kepone	0.40 ppb Kepone	0.80 pp Kepone
% to Megalopa	92	100	100	96	100	100
% to Crab	92	100	100	94	100	100
% Megalopa to Crab	100	100	100	98	100	100

SERIES II

	Acetone Control	0.05 ppb Kepone	0.10 ppb Kepone	0.20 ppb Kepone	0.40 ppb Kepone	0.80 pp Kepone
% to Megalopa	92	96	98	92	94	94
% to Crab	90	56	92	92	92	46
% Megalopa to Crab	98	60	94	100	98	52

TABLE VI

Species: Rhithropanopeus harrisii
Bowl Size: 8.9 cm diam
# of Bowls: 5 per concentration
Larvae per bowl: 0
Total # Larvae: 50 per concentration

Date hatched: 9/16/77
Salinity: 20°/oo
Temperature: 25°C
Light: 12-12
Diet: *Artemia* nauplii

SERIES III

	Acetone Control	0.1 ppb Kepone	2.5 ppb Kepone	5.0 ppb Kepone	7.5 ppb Kepone	10.0 ppb Kepone
% Survival for 11 days	100	100	96	100	100	96

SERIES IV

	Acetone Control	0.1 ppb Kepone	2.5 ppb Kepone	5.0 ppb Kepone	7.5 ppb Kepone	10.0 ppb Kepone
% Survival for 11 days	100	100	96	98	92	96

TABLE VII

Species: *Rhithropanopeus harrisii*
Bowl size: small, 8.9 cm diam
# of Bowls: 5 per concentration
Larvae per bowl: 10
Total # Larvae: 50 per concentration

Date Hatched: 9/27/77
Salinity: 20$^{o}/_{oo}$
Temperature: 25°C
Light: 12-12
Diet: *Artemia* nauplii

SERIES V

	Acetone Control	10.0 ppb Kepone	20.0 ppb Kepone	40.0 ppb Kepone	80.0 ppb Kepone	160.0 p Kepone
% Survival for 11 days	82	86	88	34	0	0

SERIES VI

	Acetone Control	10.0 ppb Kepone	20.0 ppb Kepone	40.0 ppb Kepone	80.0 ppb Kepone	160.0 p Kepone
% Survival for 11 days	86	100	90	64	0	0

The Role of Sediments in the Storage, Movement and Biological Uptake of Kepone in Estuarine Environments

Annual Report

to:

The Environmental Protection Agency

From:

Robert J. Huggett, Project Manager
The Virginia Institute of Marine Science

For the period
10/20/76 to 10/20/77

Grant Identification Number
R804993010

Preface

Included in this document are three sections which describe the efforts of the Virginia Institute of Marine Science's staff on the Role of Sediments in the Storage, Movement and Biological Uptake of Kepone in Estuarine Environments. The first section is entitled: "Kepone in James River Sediment," by Maynard M. Nichols and Richard C. Trotman. The second, "Kepone Water-Sediment Elutriates," by Robert J. Huggett and the third, "Uptake of Kepone From Suspended Sediments by Oysters, Rangia and Macoma," is by Dexter S. Haven and Reinaldo Morales-Alamo.

Also attached is a progress report on the EPA funded James River Hydrographical Survey Study which was conducted in the late summer of 1977.

KEPONE IN JAMES RIVER SEDIMENTS

An annual progress report to EPA

by

Maynard M. Nichols and Richard C. Trotman

October 1977

1. Purpose.

This study aims to determine where kepone has accumulated in the bottom sediments; that is, where are the sediment sinks for kepone? A second aim is to trace the routes and rates of transport; that is, what happens to kepone-bound sediment when released from its source? Finally, how long will it take to reduce levels of kepone in the sediment by natural processes?

Results emerging from the study are of use to advise state and federal authorities how to clean-up kepone pollution through natural processes. They provide basic data on sedimentary processes for benthic ecosystem models; they are of use for evaluating the effects of dredging kepone-rich sediments. As a tracer of sediment, kepone provides new information on sediment dispersal and the circulation of fine-grained material in a classic estuary.

2. Highlights of Activities.

Efforts during the period were highlighted by the following:

- Review of James River sediment data to predict fate of kepone for program formulation.

- Presentation of paper on results historical review, First Kepone Seminar, at VIMS, October 1976.
- Preliminary field sampling of surface sediments along length of James in three periods, September, December 1976, and March 1977; 37 to 52 stations sampled during each period; 18 cores obtained.
- Co-ordination conferences with EPA program manager, Dr. Tudor Davies, Gulf Breaze and Virginia State Water Control Board, October through December, 1976.
- Employment of project personnel, Mr. Richard Trotman, completed April 1977; sedimentologic effort in full swing.
- Liason with Battelle Northwest, Dr. Onishi, on field programs and math model formulation.
- Liason with Manhatten College, Dr. D. O'Conner and R. Thomann, concerning formulation of a math model for sediment and kepone transport.
- Development of structure for mathematical model of sediment-kepone transport with Dr. Kuo.
- Formulate plans for suspended sediment-kepone field study, May 1977.
- Follow-up sampling of bottom sediments and selected cores of dredge disposal sites, July 1977. Continued lab analyses of these samples and previous samples.

- Preparation for field study; filters, field equipment, and field labs for processing suspended sediment, June through July 1977.
- Field observations, sampling and measurement of kepone on suspended sediment, currents, and related parameters, August 1977.
- Laboratory analyses of suspended sediment samples, total concentration, organic content, September through October 1977.
- Participation in Second Kepone Seminar and kepone Symposium at the 4th International Conference on Estuaries.
- Follow-up sampling of bed sediments in Hampton Roads and lower Chesapeake Bay in conjunction with closing of area to crabbing; 12 stations occupied.
- Field sampling of bed sediments curtailed in October 1977. Data reduction largely complete.

3. Approach.

Efforts during the period mainly consisted of field sampling, laboratory analyses, and data reduction. First, historical data on kepone and James River sediments were reviewed to identify probable kepone sediment sinks and relative rates of deposition. Sampling stations were sited throughout the estuary in relation to water depth, bathymetry, oyster grounds, deposition patterns, dredge and disposal sites, and in relation to the kepone source.

Field procedures were worked out to sample freshly deposited sediment on the bed as well as in cores at selected sites. Laboratory procedures were set up to process samples for particle size and organic content. The horizontal and vertical distributions of kepone were delineated graphically and evaluated with time over one year in relation to basic information concerning sedimentary processes and transport of fine-grained sediment. An attempt was made to determine from field samples the distribution of kepone in relation to particle size and organic content.

4. Methods and Procedures.

Bed sediments were obtained by a Petersen grab with a 0.05 m^2 bite area or a 7.6 cm (3-inch) diameter corer. The corer was especially constructed for obtaining soft mud with minimal disturbance. Approximately 30 ml of sediment was obtained from the top sediment surface and returned to the laboratory for analyses. Stations were closely positioned by ranging or sextant bearings on buoys and landmarks. Samples were frozen prior to laboratory analyses.

In the laboratory bulk sediment samples were processed for: (1) kepone content, (2) organic matter by loss on ignition, and (3) particle size (percentage sand, silt and clay) by sieving and pipette. Additionally, the sieved fraction, less than 63μ of samples collected in September and December 1976, was analysed for both kepone content and for particle size by a Coulter Counter. Laboratory methods follow conventional procedures described in

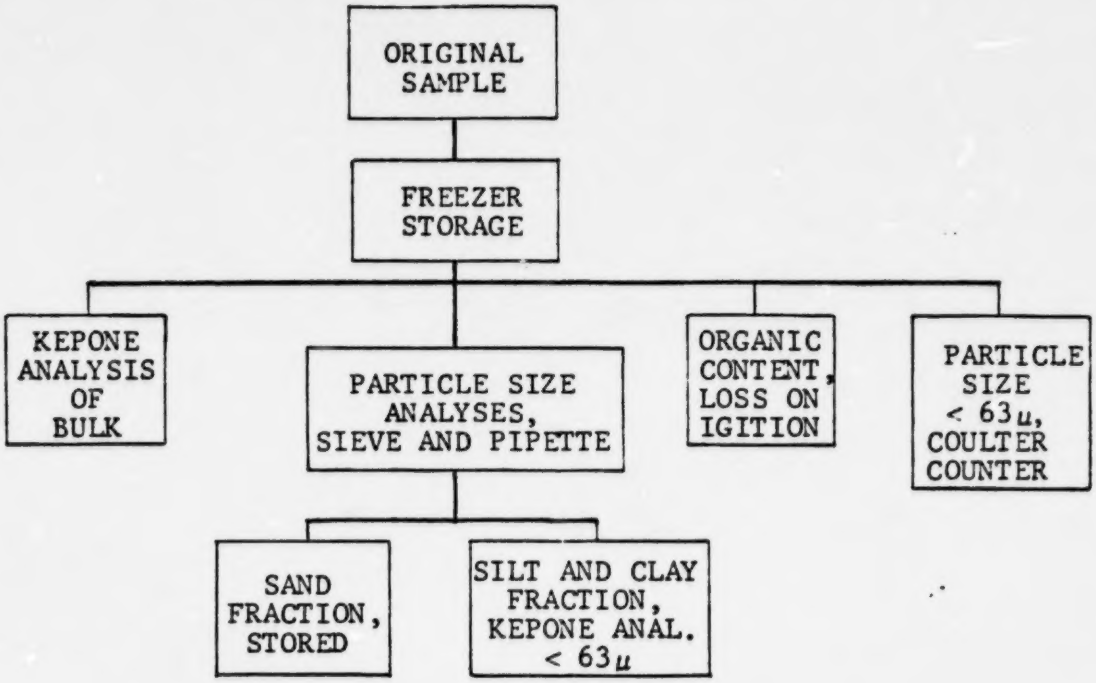
ORIGINAL SAMPLE
FREEZER STORAGE
KEPONE ANALYSIS OF BULK
PARTICLE SIZE ANALYSES, SIEVE AND PIPETTE
ORGANIC CONTENT, LOSS ON IGITION
PARTICLE SIZE < 63u, COULTER COUNTER
SAND FRACTION, STORED
SILT AND CLAY FRACTION, KEPONE ANAL. < 63u

Figure 1. Scheme for laboratory processing of bed sediments.

Moncure and Nichols (1968), Standard Methods (1973) and Folk (1961). Details are given in laboratory instructions on file at VIMS sedimentological lab. Figure 1 summarizes steps in laboratory processing.

5. Results and Their Significance.

Spatial Variability. A special study of variations in kepone concentrations in bulk bed sediment over a small spatial range was conducted at two selected stations: (1) station 15 in lower reaches near Wreck Shoal with 3 m water depth and (2) station 40a in middle reaches at buoy 62 with 6 m water depth. At station 15, four samples were taken at random from the top < 2 cm of sediment and of the top < 15 cm of sediment, all from the same grab. Table 1 lists the results. Spatial variations within the 0.05 m^2 area of the grab are relatively small with standard deviations less than ± 7 percent.

Table 1. Variation in kepone concentrations in the top < 2 cm and the top < 15 cm of sediment from a single grab; station 15, June 15, 1977.

Depth Interval	Kepone, ppm		
0-2 cm	0.026		
	0.025	Mean :	0.027
	0.029	Range :	0.025 - 0.029
	0.026	Std. Dev.:	± .002 (± 7%)
0-6 cm	0.012	Mean :	0.013
	0.013	Range :	0.012 - 0.013
	0.013	Std. Dev.:	± .001 (± 8%)

At station 40a one sample was taken of the top < 2 cm of sediment from 10 successive grabs. The grabs were obtained at random while the vessel drifted over distances of 225 m downstream and 135 m upstream from the station. Results of the sampling and analyses (Table 2) indicate a very wide range of values within a distance less than 230 m. Despite the low bottom relief and small textural differences of the sediment at the site, kepone concentrations ranged as much as 0.41 ppm. When surface samples were taken at random from 12 successive grabs at the same station, number 40a, (Table 2) (an anchor station with an area of about $200\ m^2$) the kepone concentrations ranged 0.47 ppm with a standard deviation of 44 percent.

Table 2. Spatial variation in kepone concentrations from the top < 2 cm of sediment of successive grabs at station 40a, July 5, 1977 (drift station) and July 20, 1977 (anchor station).

Drift Station		
Downstream 225 m	Upstream 135 m	Anchor Station
0.062	0.021	0.27
0.074	0.025	0.17
0.081	0.029	0.44
0.067	0.023	0.21
0.096	0.017	0.14
0.110	0.013	0.27
0.130	0.027	0.33
0.340	0.033	0.39
0.360	0.029	0.34
0.470	0.023	0.61
		0.61
		0.28
Mean : 0.179	Mean : 0.024	Mean : 0.338
Range : 0.062 - 0.470	Range : 0.013 - 0.033	Range : 0.14 - 0.61
Std. Dev.: ± 0.151 (± 84%)	Std. Dev.: 0.006 (25%)	Std. Dev.: ± 0.153 (± 44%)

The marked variations are partly due to the sampling process whereby some surface sediment is necessarily washed in the grab or disturbed at depth. However, most local variations are inherent in the bed sediments which are affected by variations in scour and fill, variations in texture and organic matter. Such variations define rather broad limits which may be placed on the kepone distribution as a function of location. They affect "seasonal" distributions inasmuch as the navigational capability of relocating a station is no better than a circle 130 m in diameter.

Distribution of Kepone in Surface Sediments. The sediments from middle reaches are the most contaminated. As shown in Figure 2, average kepone concentrations in bulk bed sediments from the channel (> 4 m depth) are higher between mile 38 and 52 than near the source (mile 63) or farther seaward in the estuary. This is the zone of the turbidity maximum which lies landward of the inner limit of salt intrusion. Suspended sediment concentrations in this zone are higher than elsewhere most of the year.

When longitudinal distributions of kepone are compared for surveys in December 1976, March 1977, and July 1977, there are no significant trends with time. Instead the concentrations are relatively stable within a range of about 0.10 ppm. However, the average levels of concentration from December 1976 through July 1977 in middle reaches (0.15 ppm) are generally

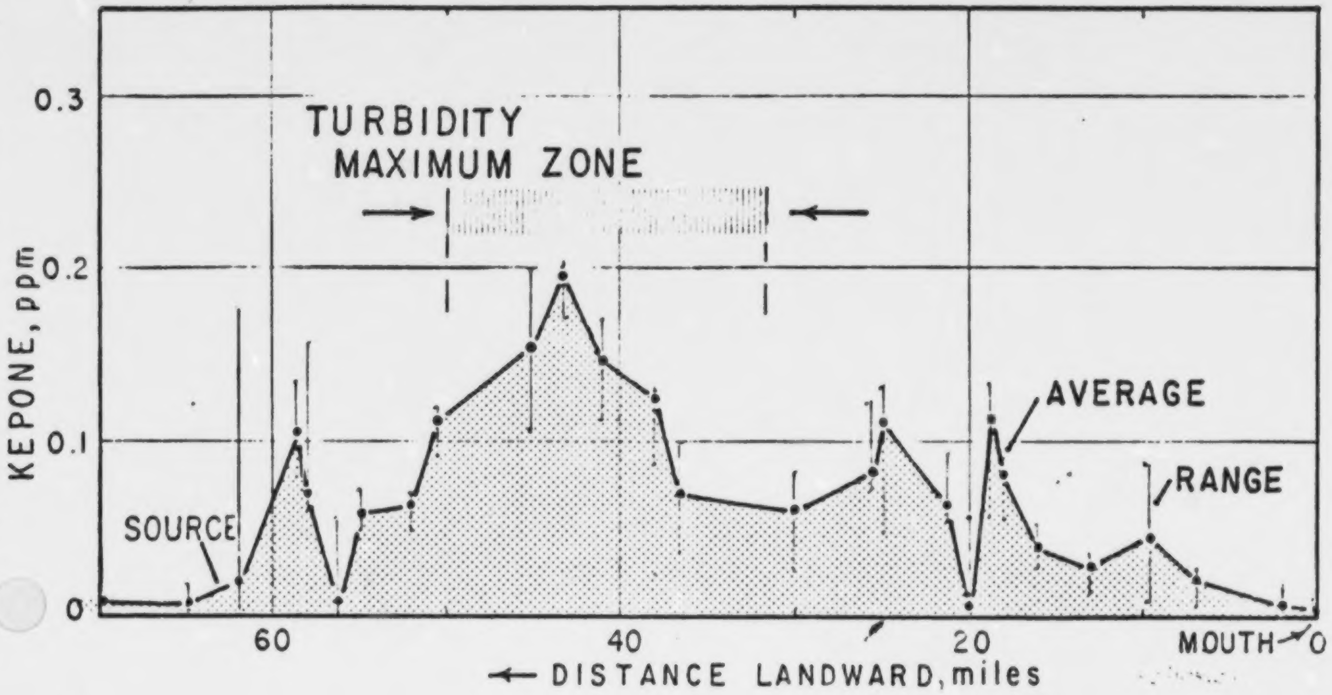

Figure 2. Longitudinal distribution of average kepone concentrations in bed sediments from the channel of the James Estuary; mean of December 1976, March and July 1977 values.

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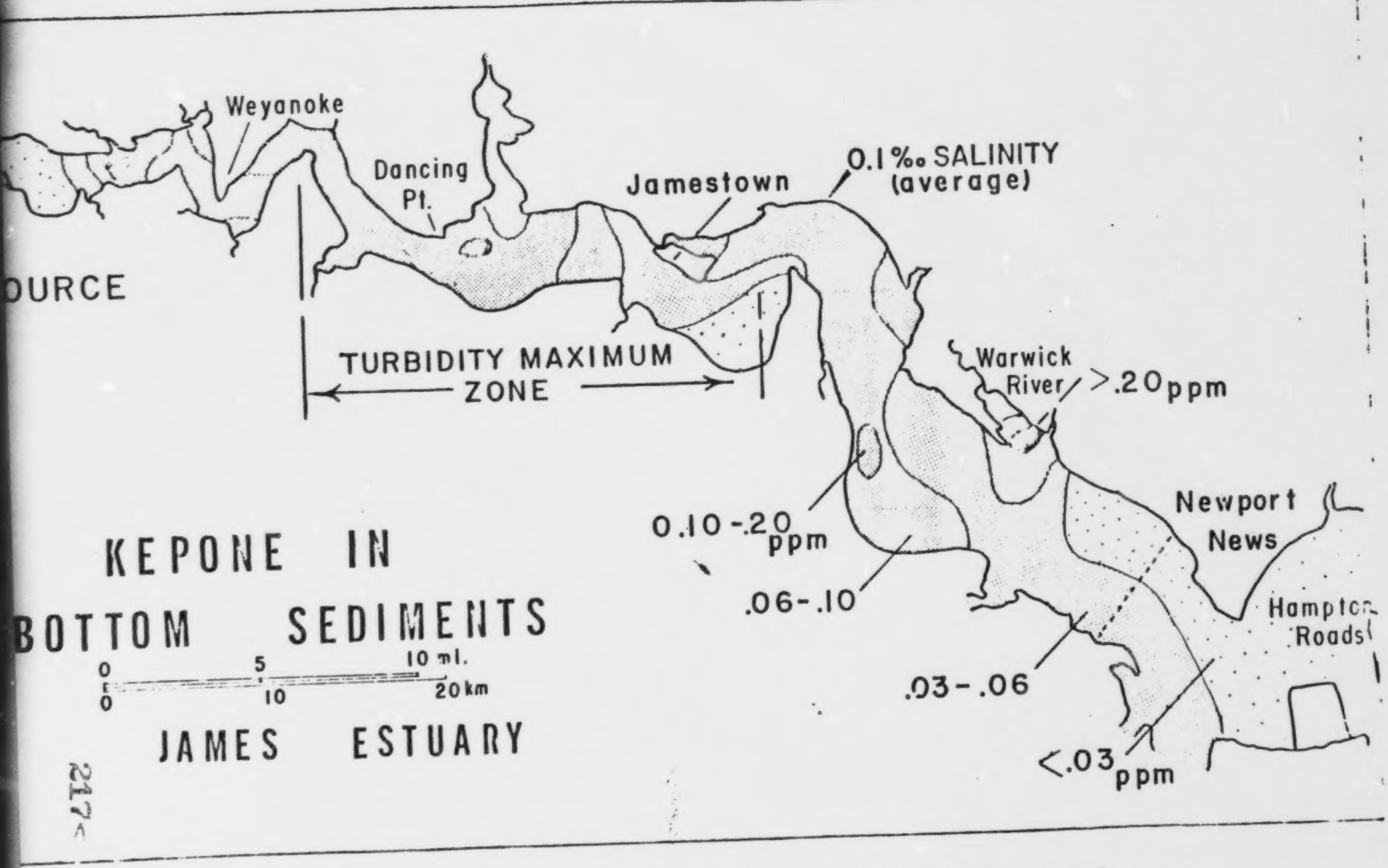
Weyanoke
Dancing Pt.
Jamestown
0.1‰ SALINITY (average)
OURCE
TURBIDITY MAXIMUM ZONE
Warwick River
>.20ppm
Newport News
Hampton Roads
0.10-.20 ppm
.06-.10
.03-.06
<.03 ppm
KEPONE IN
BOTTOM SEDIMENTS
0 5 10 mi.
0 10 20km
JAMES ESTUARY

Figure 3. Horizontal distribution of average kepone concentrations in bed sediments; mean of December 1976, March and July 1977 values.

lower than those measured earlier by VIMS in September 1976 and by the Corps of Engineers in January 1976 when concentrations were 0.27 to 0.48 ppm.

The zone of high sediment contamination covers both channels and contiguous shoals. As shown in plan view, Figure 3, average concentrations are higher in the reach between Jamestown and Weyanoke than elsewhere. The highest average concentration is in sediment from a shoal off Dancing Point. Elsewhere, concentrations are locally high off mouths of tributary creeks such as Bailey's Creek near the kepone source, Chippokes Creek, The Thorofare, Jamestown and the Warwick River. Substantial concentrations, ranging 0.66 to 1.20 ppm, are found in Burwell Bay. However, concentrations are relatively low in narrowed reaches around Hog Point. Kepone content generally diminishes seaward from Burwell Bay to Hampton Roads where concentrations are less than 0.010 ppm. Twelve sediment samples from lower Chesapeake Bay in September 1977 all had concentrations less than 0.010 ppm.

Distribution of Kepone at Depth in Sediments. Contamination of bed sediments in zones of natural fill (undredged) extends to about 40 cm below the bed surface (Figure 4). Greatest contamination, often exceeding 0.50 ppm, occurs at depths of 10 to 20 cm below the surface. However, in cores from shoals in the shipping channel where sedimentation is locally fast (i.e., 30a), concentrations increase downward to a depth of 60 to 80 cm. This trend reflects the diminished supply of kepone-rich sediment with

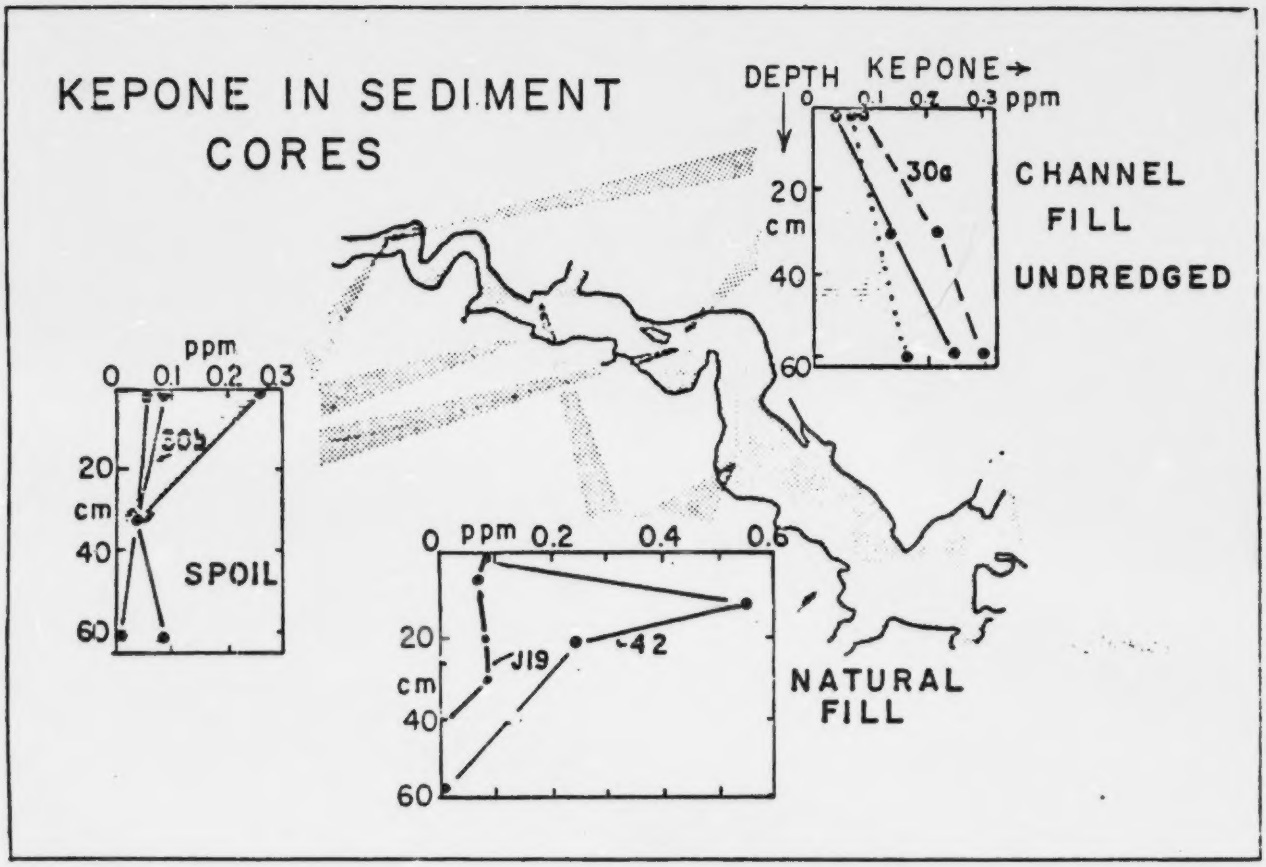

Figure 4. Depth distribution of kepone in cores from selected sites.

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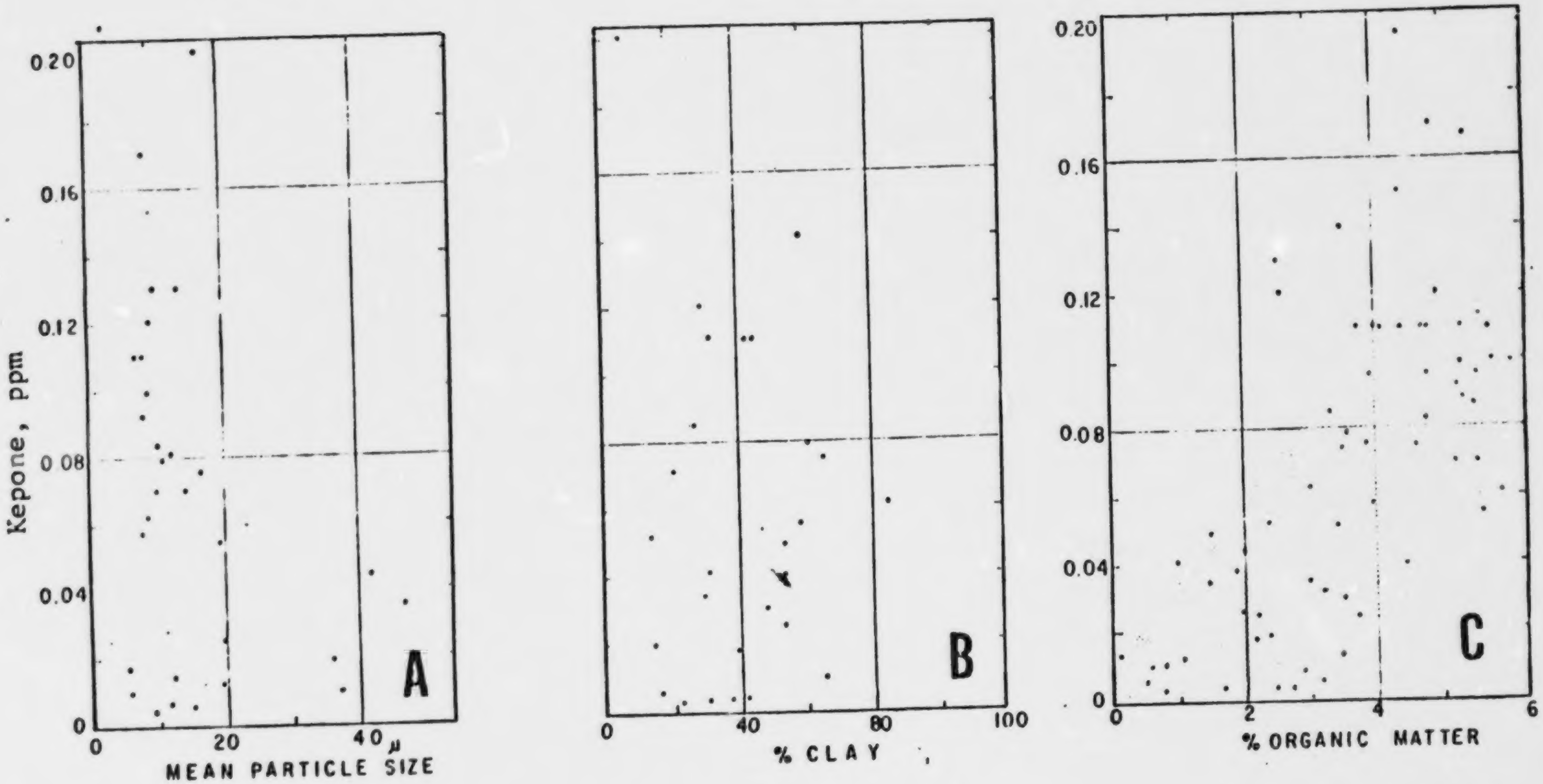
time since the Summer of 1975. Kepone content of old dredged material decreases slightly with depth (i.e., cores 41a, 30b, 1.9-7.5). The depth trend results from mixing of sediment during dredging and disposal. The contaminated sediment is most likely mixed and "diluted" by uncontaminated sediment and thus reduces the overall concentration.

A few samples from the Jamestown-Dancing Point reach collected in May 1967 showed dectable amounts of kepone (.038 and .018 ppm). Although the content is low, the samples suggest that the life span of kepone in the sediments is at least 10 years.

State of Kepone in Sediments. The concentrations of kepone are orders of magnitude greater in the bed sediments than dissolved in estuary water. An indication of the state of kepone storage in the sediments is gained by examining its relation to percent clay content, mean particle size and organic content.

Finer-grained sediments are generally the most contaminated. A plot of mean grain size versus kepone concentrations throughout the estuary (Figure 5a) shows a great deal of scatter. Likewise a plot of percent clay content versus kepone concentrations varies widely (Figure 5b). Part of the scatter results from the great variation in textural types throughout the estuary whereas kepone content partly varies in relation to its source. When kepone content of samples from a single reach of the estuary is considered, however, there is a trend for higher kepone content in the fine-grained sediment with high clay content.



igures 5a. Kepone content versus mean particle size.

5b. Kepone content versus percent clay content.

5c. Kepone content versus organic matter indicated by loss on ignition.

There is a distinct trend of increasing kepone content with increasing organic content. As shown in Figure 5c, organic-rich sediments have higher kepone content than sediments with low organic content. As expected, samples landward from the kepone source or from zones of scour, display wide scatter. The trend indicates kepone prefers organic matter, either adsorbed on detrital particles or ingested when the organic matter was produced. As organic matter slowly decomposes in the sediment, there is an opportunity for kepone to escape into interstitial or overlying water.

6. Discussion.

Sedimentary Sinks for Kepone. The James Estuary is an environment where much river-borne sediment accumulates. Zones of active deposition may be expected to be areas of relatively high sediment contamination. On the other hand, zones where the bed is scoured into older sediment or zones where river-borne sediments are by-passed, are zones of relatively low contamination. Inasmuch as sedimentary processes are relatively slow, deposition sites are indicators of long-term processes. They are an end product of short-term variations induced by local wave and current transport.

Kepone contamination is generally greatest in sites of active sedimentation: (1) the Jamestown-Dancing Point reach which is also the site of the turbidity maximum, (2) Burwell Bay, and (3) tributary creek mouths. Zones of sedimentation have been

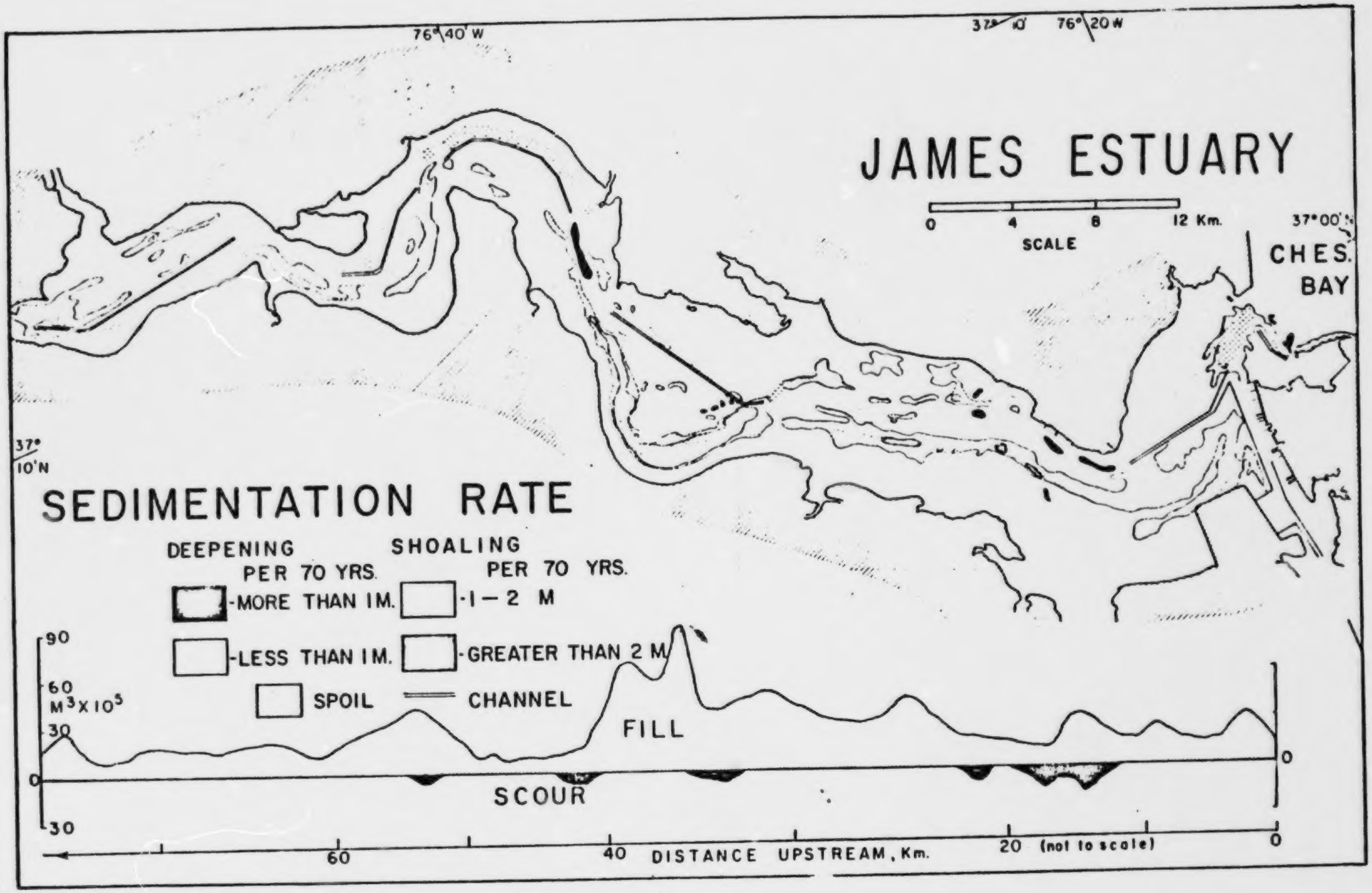

JAMES ESTUARY
0
4
8
12 Km.
SCALE
CHES. BAY
76°40' W
37° 10'
76° 20 W
37° 00'
37° 10' N
SEDIMENTATION RATE
DEEPENING PER 70 YRS.
-MORE THAN 1M.
-LESS THAN 1M.
SPOIL
SHOALING PER 70 YRS.
-1 – 2 M
-GREATER THAN 2 M
CHANNEL
90
60
M³ X 10⁵
30
0
30
FILL
SCOUR
60
40
DISTANCE UPSTREAM, Km.
20
(not to scale)
0

Figure 6. Sedimentation rates in the James Estuary based on water depth changes over 70 years, from Nichols (1972).

delineated in a former study (Nichols, 1972) (Figure 6) from differences in water depths over 35 and 70 years. The rates of sedimentation within the zones probably change with time but the sites of deposition persist.

Kepone concentrations are locally high off the mouth of Bailey's Creek, the kepone source. However, the main distribution does not display decreasing concentrations with distance away from the source. Instead, the main sink is in the middle estuary, the zone of the turbidity maximum where suspended sediments are trapped and deposited. Sediments in this zone are finer-grained than elsewhere, less than 8μ mean size. Clay content in this zone is also higher than elsewhere in the estuary.

Routes of Transport. From the sedimentation patterns, kepone distributions and existing hydraulic knowledge of the James, it is possible to sketch the probable route of kepone-sediment transport. Both the source of kepone and the major source of suspended sediment come from the same direction, landward or upstream of the estuary. Since the influx of sediment from Bailey's Creek is very small in proportion to the influx of sediment from the main river, it is probable the kepone was mainly introduced in the dissolved form and bound to suspended sediment from the main river. Since the estuary is fresh above Jamestown most of the year, net transport from Hopewell to Jamestown is directed seaward. When suspended sediment reaches the Jamestown area, transport is slowed down because net velocity approaches zero in the null zone at the salt intrusion head. The null zone acts as a dynamic barrier that restricts seaward transport of river-borne suspended sediment carried near the bottom. Only sediment carried near the surface is transported farther seaward through the upper layer. If this sediment settles downward, it is carried back upstream to the null zone by landward density currents through the lower estuarine layer. However, sediment carried over the shoals may escape the estuary through the upper layer especially during floods like Agnes. Nonetheless, the bulk of the sediment load is trapped landward of the null zone. As a tracer of sediment, kepone supports this fact. Most kepone concentrations are located in or above the null zone and they persist with time, both over the short-term, 8 months of sampling, and over the long-term as demonstrated from the distributions at depth in cores. The data indicate that it will take a long time, many years, to reduce levels of kepone in the sediment by natural processes of decay and dispersal. Part of the kepone will be buried by "new" sediment but the most significant reduction will come by "dilution" with uncontaminated sediment introduced during freshets and floods. This trend has already started on the floor of the shipping channel where sedimentation is locally fast.

KEPONE WATER-SEDIMENT ELUTRIATES

Many pollutants have an affinity to sediments which is governed by the surface charges on particles. This is particularly true for some of the trace metals - such as zinc - with the clay mineral portion of the sediments. The magnitudes of the surface charges are affected by pH and salinity (Parks, 1967). Therefore, it was necessary to determine if Kepone behaved in a similar manner because, in the James River, both the estuarine and the tidal fresh water portions with their wide ranges of pH and salinity were contaminated by the pesticide. As well the distribution of Kepone in the bottom sediments of the James show a marked increase in that portion usually in the vicinity of the freshwater - saltwater interface. At this boundary the waters change from fresh, (salinity <0.5‰) to saline, (salinities 0.5‰ to 20 - 25‰). Also in this region the pH of the water increases from near 7 to 8 due to the buffering capacity of seawater. With these abrupt changes in pH and salinity coinciding with the change in Kepone concentration, it appeared possible that fresh water sediments, highly contaminated with the pesticide, were being "extracted" by estuarine waters as they traversed this boundary progressing seaward or that Kepone in solution was not adsorbed by sediments in saline waters. Therefore, experiments were conducted in the laboratory to determine the extractibility of sediment-Kepone by waters with varying ranges of salinity and pH.

The experimental design included two phases. The first phase was to determine the accuracy and precision of the analysis of water for dissolved Kepone and the second phase was to determine the amount of Kepone removed from contaminated freshwater sediments by waters with pH's ranging from 6 to 9 and salinities of <0.5‰ and 20‰.

These ranges of pH's and salinities bracket those found in the James River.

Phase I, Water-Kepone Analysis

The method utilized for the Kepone-water extraction was one developed by The Environmental Protection Agency, Research Triangle Park (1975). It involves liquid extraction using benzene as the organic solvent. The extractions are carried out in seperatory funnels with 3 successive treatments of the same water with benzene at a ratio of 1:10 benzene to water. The extracts are combined and then dried by passing them through anhydrous sodium sulfate. The combined extracts are then analyzed by electron capture gas chromatography.

To check the efficiency and accuracy of the procedure, Kepone free water, (obtained either from Kepone noncontaminated estuaries such as the York or from laboratory deionized-double distilled stocks), was spiked with known amounts of Kepone, extracted and analyzed (Table I).

Phase II, Water Extraction of Kepone From Sediments.

The experimental design for this phase involved subjecting Kepone contaminated sediments from the James River, obtained from the fresh water portion, near Hopewell, to waters with varying pH's and salinities. The salinities were either fresh, (0.06‰), obtained from the James River or saline, (19.5‰), gotten from the mouth of the York River at the Virginia Institute of Marine Science's facility. The pH's of these waters were adjusted to the desired levels by addition of either reagent grades of hydrochloric acid or sodium hydroxide.

After the desired pH and salinity were achieved, a portion of wet sediment (100 g) was placed in a flask and the water (250 ml) was added and the mixture was agitated with a Wrist-Action Shaker for 1 hr. Following this the sediments were separated by centrifugation and the supernatant water was extracted for dissolved Kepone by the method previously described in the Phase I section of this report.

In all, 36 separate extraction were analyzed and the resulting water Kepone concentrations were compared to that in the exposed sediments. The comparisons are reported as the percent removed by a water of a given pH and salinity in Table II.

Discussion:

The data from Phase I clearly show that the Benzene method of extracting Kepone from water yields approximately 85% or better of the amount of the pesticide from solutions spiked at 1 ppb to 10 ppb. However, at concentrations below 1 ppb the efficiency drops greatly - for instance, 64% yield at 0.5 ppb. These yields can be used to judge the accuracy obtained for Kepone analyses of water by this method. The precision estimates can be seen from the standard deviations which show ± 14% or better for spiked solutions of 1 to 10 ppb. The precision of the method for concentrations of 0.5 ppb are in the same range which suggests that a portion of the "spike" may be sorbed to the walls of the glassware or lost by some other means.

Attempts were made to try solvents other than benzene, for extraction, (ethyl acetate - toluene, methylene chloride) but with the similar results - dissolved Kepone at concentrations less than 1 ppb may be 100% in error.

Since only at the 10 ppb Kepone concentration were the effect varying salinities on the analysis compared, it is risky to judge salinity effects on the method. Evenso, there is no obvious effect using natural waters of 0.06 and 19.5%.

The extraction experiments, the results of which are given in Table II and Figure 1, show that there is no apparent affect of either salinity or pH, within the ranges used which approximate those found in the James River, on the extractibility of Kepone from sediments by water. It must be kept in mind, however, that the amounts of Kepone extracted were in the tenths of ppb range. Since the analytical methodology is less than ideal at these concentrations some differences could go undetected. Figure I shows that all results are within 2 standard errors of each other which implies no difference at the 95% confidence interval.

The data indicate that, if the analyses are correct, the partitioning coefficient of Kepone from sediment to water is approximately 6×10^{-4}, irrespective of natural ranges of pH and salinity. It follows then, that the relatively high concentrations of Kepone at the fresh water-salt water interface and upstream are likely due to the turbidity maximum (mentioned in the sediment section) rather than chemical factors such as partitioning.

References

Environmental Protection Agency, 1975, Preliminary Report on Kepone levels from Hopewell, Va area. Briefing at Research Triangle Park, North Carolina

Parks, G. A., 1967. Aqueous Surface Chemistry of Oxides and Complex Oxide Minerals: Equilibrium Concepts in Natural Water Systems, p. 121-160. In Gould, F. (Ed), Advances in Chemistry, Series 67. American Chemical Society Publications.

TABLE I

Extraction Efficiencies of Kepone from Water by the Benzene Method

Salinity	Adjusted pH	Spiked Kepone Concentration	% Recovery
0%, Deionized H_2O	7.0	10ppb	96%
"	"	"	99%
"	"	5ppb	87%
"	"	"	90%
"	"	"	78%
"	"	"	94%
"	"	"	95%
"	"	1ppb	97%
"	"	"	72%
"	"	"	93%
"	"	"	69%
"	"	"	93%
"	"	"	56%
"	"	"	102%
"	"	"	85%
"	"	"	86%
"	"	"	96%
"	"	"	83%
"	"	0.5ppm	72%
"	"	"	51%
"	"	"	67%
"	"	"	71%
"	"	"	67%
"	"	"	55%
"	"	"	80%
"	"	"	48%
"	"	"	69%
0.06% James R. H_2O	7.0	10ppb	86%
"	"	"	99%
"	"	"	92%
"	"	"	85%
"	"	"	83%
"	"	"	77%
"	"	"	76%

TA E I (continued)

Salinity	Adjusted pH	Kepone Spiked Concentration	% Recovery
19.5% York R. H_2O	8.0	10ppb	74%
"	"	"	85%
"	"	"	73%
"	"	"	99%
"	"	"	74%
"	"	"	103%
"	"	"	99%

TABLE I

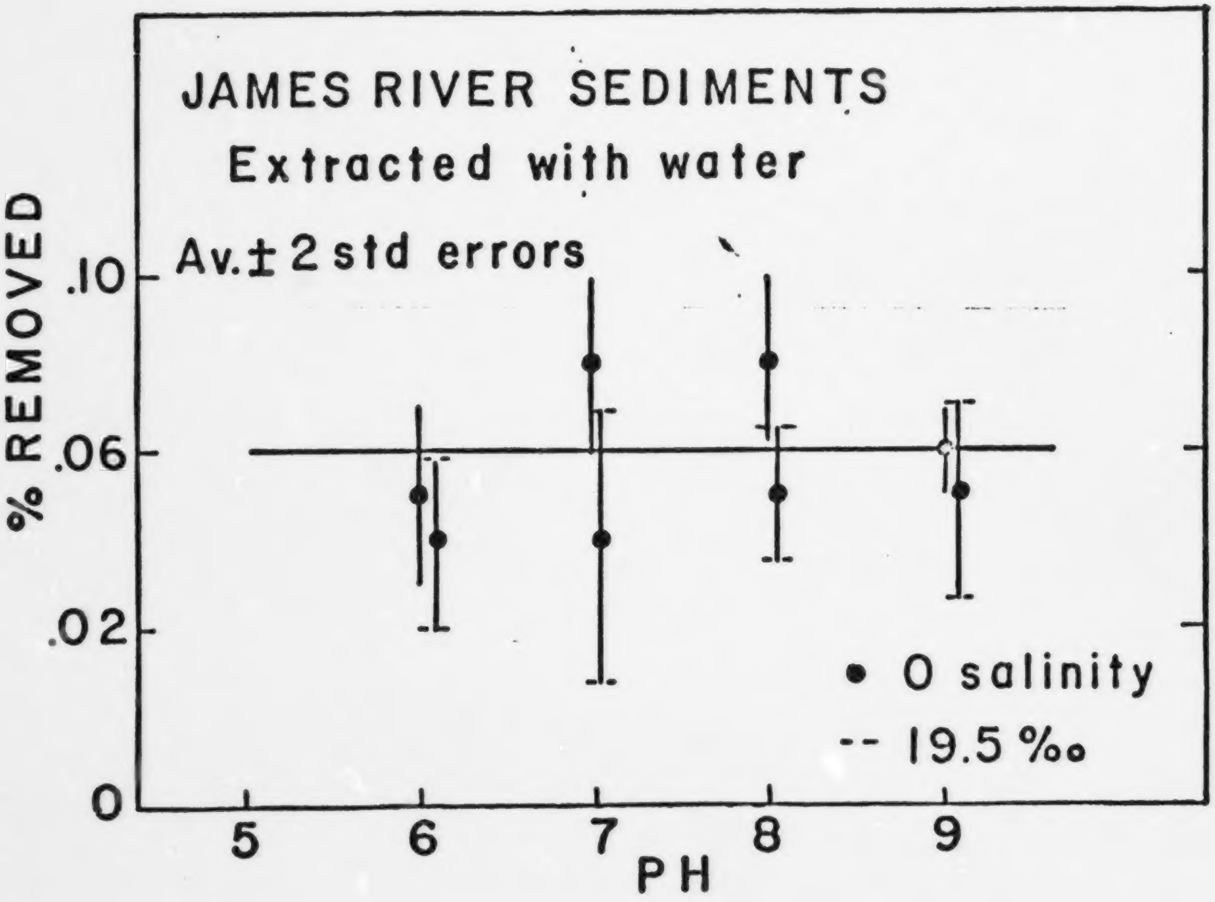
Summary

Salinity	Adjusted pH	Kepone Spiked Concentration	Average yield And Standard dev.
Deionized + Distilled	7.0	10ppb	98 ± 2%
"	"	5ppb	89 ± 7%
"	"	1ppb	85 ± 14%
"	"	0.5ppb	64 ± 11%
0.06% James River H_2O	7.0	10ppb	85 ± 8%
9.5% York River H 0	8.0	10ppb	87 ± 13%

Elutriate Results

Salinity	(Sediment ± ppm Kepone) pH	% Removed	± Sd
0.06‰	6.0	0.04%	STD. ERROR - 0.01
"	"	0.06%	0.05 ± 0.01% of total Kepone in sediment recovered at a pH 6.0 + 0.06‰
"	7.0	0.11	
"	"	0.11	
"	"	0.09	
"	"	0.12	0.08 ± 0.04% of total Kepone in sediment recovered at a pH 7.0 + 0.06‰
"	"	<0.01	
"	"	0.07	
"	"	0.06	
"	"	0.06	STANDARD ERROR 0.01
"	"	0.11	
"	8.0	0.06	
"	"	0.09	0.08 ± .02% of total Kepone in sediment recovered at pH 8.0 + 0.06‰
"	"	0.09	STANDARD ERROR 0.01
"	9.0	0.05	0.06 ± 0.01% of total Kepone in sediment recovered at pH 9.0 + 0.06‰
"	"	0.06	STANDARD ERROR 0.005
19.5‰	5.0	0.03	0.03 ± ? %
"	6.0	0.04	0.04 ± 0.02% of total Kepone in sediment recovered at pH 6 + 19.5‰
"	"	0.06	
"	"	0.03	STANDARD ERROR 0.009
"	7.0	0.02	STANDARD ERROR 0.015
"	"	0.07	0.04 ± 0.03% of total Kepone in sediment recovered at pH 7 & 19.5‰
"	"	0.04	

"	8.0	0.09	
"	"	0.06	
"	"	0.05	
"	"	0.06	0.05 ± 0.02% of total Kepone in sediment recovered at pH 8.0 + 19.5‰
"	"	<0.01	
"	"	0.05	
"	"	0.04	
"	"	0.06	STANDARD ERROR 0.007
"	"	0.02	
"	"	0.05	
"	9.0	0.07	0.05 ± 0.02% of total Kepone in sediment recovered at pH 9 + 19.5‰ STANDARD ERROR 0.012
"	"	0.05	
"	"	0.03	


JAMES RIVER SEDIMENTS
Extracted with water
Av.± 2 std errors
% REMOVED
.10
.06
.02
0
5
6
7
8
9
PH
● 0 salinity
-- 19.5 ‰

UPTAKE OF KEPONE FROM SUSPENDED SEDIMENTS BY OYSTERS, RANGIA AND MACOMA

Introduction

Laboratory studies on the uptake of Kepone from sediments in suspension by bottom-dwelling organisms were undertaken by the Virginia Institute of Marine Science at Gloucester Point, Virginia on December 1, 1976. The first two months were spent in acquisition and preparation of laboratory equipment and space for the experiments.

In the period of time since then, three series of laboratory experiments were conducted with three species of bivalves. Eight experiments were completed with the oyster Crassostrea virginica, five with the clam Rangia cuneata and one with the clam Macoma balthica. Most of these experiments involved exposure of the animals to contaminated sediments in suspension. In two of them, however, the animals were placed in a bed of contaminated sediments with uncontaminated river water flowing over them.

This report presents the results of three series of experiments followed by a discussion.

Materials and Methods

Apparatus

A diagram of the basic arrangement of the apparatus used to conduct these experiments is shown on Figure 1. The units labelled A through D were used only during the first series of experiments when ambient river water temperature was below 10 C most of the time. York River water was piped into a constantly-overflowing box (A) from which it was pumped through heat exchangers (C) into a rectangular cascading trough (D). The latter served to allow bubbles created by the escape of dissolved gases to dissipate before reaching the animal trays. This section of the system was not used in the last two series of experiments when river water temperatures were above 10 C. Then, York River water was piped directly into a rectangular trough (E) which was suspended from the ceiling directly above the wet table that held the experimental trays. Water depth in the trough was maintained at 20 cm by a drain standpipe of that height.

Water to supply the experimental trays was siphoned out of trough E with plastic tubing. In the first series of experiments water flow rates were controlled by inserting glass flowmeters (F) in the tubing siphons ahead of the mixing chambers (I). In the last two series of experiments the flowmeters were omitted. Instead, flows were regulated by the bore size of the plastic tubing used for siphons. This eliminated constrictions in the tubing caused by adjustable clamps which enhanced flow interruptions due to clogging. Siphons were cleaned daily and flow measurements made before and after the siphons were cleaned.

Water from the siphons entered a rectangular mixing chamber made of acrylic plastic (I), 25 cm in length, 16 cm in width and 14 cm in height, through a smaller chamber (2 cm long, 3.5 cm wide and 14 cm high). The smaller chamber was connected to the larger one by a circular opening with a 2 cm diameter. Contaminated sediment suspensions also entered the mixing chamber through the same small chamber. Stock suspensions were kept well mixed in flasks (H) by magnetic stirrers (J). They were metered into the mixing chamber at a constant rate by peristaltic pumps (G).

River water and sediment suspensions were mixed in the mixing chamber by magnetic stirrers. Observation showed that the mixing was complete before the mixture flowed out of the mixing chamber. Sedimentation in the chamber was negligible. The diluted sediment suspensions flowed into the experimental trays (K) through a standpipe located at the end opposite to the one through which water and sediments entered the chamber. The system set up was the same for trays holding control animals except for elimination of components G and H.

In experiments with the clam *Rangia cuneata*, York River water salinity was reduced to between 5 and 6^{o}/oo by addition of fresh ground water pumped from a shallow well. A second rectangular trough (P) was suspended below the one receiving York River water (E). York River water was siphoned (Q) from trough E into trough P. Fresh water was also piped into a cascading trough similar to D to eliminate gas bubbles generated by the change in pressure the ground water was subjected to before it flowed into trough P. Water of the resulting lower salinity was then siphoned into the trays holding *Rangia* clams following the same system setup labelled F through K in Figure 1.

Figure 2 shows a partial view of the apparatus used in the series of experiments.

A system of sediment traps was used to insure that no contaminated sediments from our experiments escaped into the floor drain which emptied into the York River. The first component was the wet table on which the experimental trays were set (L in Figure 1). A standpipe about 2.5 cm high inserted in the drain hole of the wet table converted the table into a sediment trap. A plastic circular tank (50 cm high and 30 cm in diameter) received water from the wet table through a pipe reaching close to the bottom. The tank overflowed near its top into a series of three rectangular boxes (114 cm long and 25 cm wide), each with a 15 cm high standpipe overflow. The third box overflowed into the floor drain. The sediments and other excess solids obtained in the experiments were collected in carboys for disposal.

Experimental Trays

Two types of trays were used to hold experimental animals. In most experiments, a tray made of acrylic plastic 49 cm long, 26 cm wide, and 8 cm high, were used. The overflow end was 6 cm high and that also was the depth of the water in the tray. This tray was not compartmentalized and the animals laid directly on the bottom (Figure 3).

A larger acrylic plastic tray, 81 cm long, 54 cm wide and 8 cm deep was used in the third series of experiments to hold oysters whose biodeposits were collected. A baffle at the overflow end of the tray maintained water level at a depth of 6.5 cm. These trays were divided into 25 compartments by plastic strips 2.5 cm high. Each compartment held one oyster. The compartments facilitated separation and collection of biodeposits.

Biodeposits

Biodeposits produced by oysters receiving contaminated sediments in suspension in the large trays were collected every day with a bulb pipette. The aggregates collected at the end of each weekly period were then analyzed for Kepone. Every time biodeposits were collected, sediments settling out by gravity in the same tray were also collected and the weekly accumulation also analyzed for Kepone contents. Each day, after biodeposits and sediments had been collected, every compartment was cleaned of any remaining sediments.

Animals Buried In Mud

A modification to the manner usually used to expose animals to contaminated sediments, i.e., by flowing sediment suspensions over them, was introduced in the third series of experiments. Oysters and Rangia were buried partially and fully, respectively, in beds of contaminated sediments held in the smaller of the trays described above (Figure 4). The sediment bed was 4 to 5 cm deep. It was made up of unsieved sediments from the same batch used in simultaneous experiments with flowing suspended sediments.

Oysters were pressed into the sediments at about a 30° angle. Up to one-third of their height was below the sediment surface level. The valve area over the gills protruded above the sediment surface. Rangia were pressed into the mud so that almost the whole animal was below the sediment surface level. Within several hours they had buried themselves fully into the sediment so that only their siphons showed. Water flowing over the animals and the sediment bed had no sediments added to it and was approximately two to three cm deep.

Source of Experimental Animals

The animals used were obtained from areas to be free of Kepone^R. Rangia and Macoma were collected from the Rappahannock River and oysters came from the Piankatank River. All three species were acclimated to the experimental temperatures and salinities under flowing-water conditions at least one week prior to use. Analysis before start of each experiment showed them to be free of contamination with Kepone^R.

Preparation of Sediment Suspension

Figure 5 presents a flow chart outline of the steps taken in preparation of Kepone^R contaminated sediment suspensions. All contaminated sediments were collected with a sediment grab sampler at Jordan Point, in the James River at Hopewell and represented the top 6 cm of the bottom. They were transported to the laboratory in 2 or 3 large plastic bags each containing about 20 kg of material. The contents of each bag was mixed and transferred to smaller bags in fractions of approximately 500 ml in volume. The smaller bags were stored in a freezer until needed. Only sediments collected on the same date were used in any one series of experiments.

When needed, a bag of sediments was thawed, mixed with well water and shaken mechanically in flasks for 12 hours or more. The sediments were then wet-sieved through a 63 u and the resulting suspension diluted up to 7000 ml with well water. This volume was labelled as stock suspension and given an identification number. It was maintained in suspension by continuous agitation with a magnetic stirrer and bar. Subsequently, to insure homogeneity in dosage, it was divided into measured portions by alternately siphoning a small volume into each of six containers and repeating the cycle until each container had been filled to the desired volume.

The samples in two of the containers, with volumes of approximately 400 and 200 ml, were used to determine the concentration of Kepone in the suspension and the dry weight per unit volume of the sediments in the suspension, respectively. The suspension in the other four containers, usually with volumes of 1200 and 1600 ml, was the material to be introduced into the trays holding experimental animals. The suspensions in the four containers were diluted in a ratio of 1:4 and pumped into the mixing chambers.

Sampling of Animals in Trays

Samples of the animals were analyzed for Kepone at the start of each experiment and at approximately weekly intervals thereafter for the four-weeks duration of the experiment. Each sample consisted usually of three or four animals and at times of up to eight individuals in the case of oysters and Rangia. In the case of Macoma the number ranged between seven and fifteen. The shell of each animal was carefully scrubbed after removal from the tray.

Kepone Analysis

Analysis of all samples from concentration of Kepone were done by personnel of the Department of Ecology and Pollution in their laboratories. The method used was soxhlet extraction, fluorosil cleanup and electron-capture gas cromatography.

Determination of Kepone Concentration in Sediments

The concentration of Kepone in the diluted sediment suspension flowing over the experimental animals was determined by computation of the product of four factors:

$$K_c = (s_c)\ (k_c)\ (d_1)\ (d_2)$$

where

K_c = computed Kepone concentration in diluted suspension, in ppb

s_c = sediment dry weight per unit volume in stock suspension, in Kg/l

k_c = Kepone concentration determined analytically

d_1 = factor by which stock suspension was diluted prior to being pumped into mixing chambers.

d_2 = factor by which the suspension being pumped into mixing chambers was diluted; determined by the flow rate at which it was being pumped and the flow rate of York River water flowing simultaneously into the mixing chamber.

The factor d_2 was controlled in each experiment by selection of peristaltic pump settings that would deliver a desired flow rate of the sediment suspension into the mixing chamber. The flow of river water into the mixing chamber was also adjusted to the desired rate. Flow of river water was maintained relatively constant while the flow rate of contaminated sediments was adjusted so that trays would receive sediment suspensions at different rates.

Some trays received what was labelled as low concentrations of sediment (and, therefore, also of Kepone) while others received medium and high concentrations. Throughout an experiment the ratio between low, medium and high concentrations remained fairly constant even though Kepone concentrations in stock suspensions were variable. As a result, the concentrations labelled as low were always significantly lower than those labelled medium or high. The separation of low concentrations from medium and high ones is the main distinction made between concentrations in this report.

Preparation of Data for Analysis

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In the course of one series of experiments between 30 and 40 different stock suspensions (500 ml bags) were used.

Sediment concentration and Kepone concentration varied from one stock suspension to another. Consequently, the experimental animals in any one tray were not being exposed to a constant concentration of Kepone during the time they were held in the trays. However, throughout the duration of an experiment, the ratio between low, medium and high concentrations remained fairly constant. As an aid in interpretation of results, a weighted mean hourly concentration was computed for each of the weekly periods as the sum of the products of the concentration in each stock suspension and the length of time (in hours) that particular suspension was used, divided by the total number of hours in the weekly period. Included in these computations, were short intervals during which, for a variety of reasons, no sediments were being added to the water flowing over the animals. These intervals were usually few and anywhere from 15 min to 2 hour in duration. Also involved was a final interval of eight to nine hours at the end of a weekly period when the animals only received river water to allow them to eliminate material held in their digestive tract.

Despite the mixing done before the sediment sample was divided into 500 ml fractions, differences in sediment and Kepone concentrations from one stock to another were sometimes large. Thus, differences of significant magnitude were encountered sometimes between the mean hourly concentrations for the different weekly periods in one experiment. Since there was a high correlation between the concentration of

Kepone in oyster meats and its concentration in the sediments, and to eliminate the effect of the variations between stock suspensions, the values for oyster meats were normalized by re-computation based on the mean hourly concentration of Kepone in the sediments over the approximately four-weeks duration of experiment. The new values represent the concentration of Kepone expected in oyster meats if the concentration in sediments was constant. The normalized values were computed using a proportional equation.

Results

Fourteen uptake experiments were completed between February and August 1977. Eight with the oyster Crassostrea virginica, five with the wedge clam Rangia cuneata and one with the clam Macoma balthica. In all but two of these, the animals were exposed to suspended contaminated sediments in flowing water. In the other two, oysters and Rangia were exposed to contaminated sediments by partial or total burial in an undisturbed bed in a tray.

Exposure periods consisted of approximately one to four weeks. Kepone concentrations in the sediment suspension were computed for each weekly period and for each of the progressively longer periods that represented the total duration of exposure.

Kepone levels in the sediment suspensions were classified as low, medium and high as labels of convenience. The mean hourly concentration for each weekly period in experiments where levels were classified as low ranged between 0.020 and 0.058 ppb (Tables 1-3). In experiments where levels were classified as medium and high, mean hourly concentrations ranged between 0.040 and 0.153 ppm.

Mean hourly concentrations for the total duration of exposure (one, two, three or four weeks) in experiments where levels were classified as low ranged between 0.027 and 0.058 ppb (Tables 4 and 5). In experiments where levels were classified as medium or high the range of mean hourly concentration was between 0.057 and 0.153 ppb.

Results are presented separately for each of the three bivalve species. No data are presented for the Kepone concentration in animals examined before the start of each experiment or for control animals because in every case they were under the level of detectability of the analytical procedure.

Crassostrea virginica

Figures 6-8 show the concentration of Kepone in oysters examined at weekly intervals after exposure to contaminated sediments in suspension in three series of experiments. The values in parentheses give the mean hourly concentration of Kepone in the sediments for the weekly period that immediately preceded removal for analysis of that particular sample of oysters.

Results of the first series of experiments showed a uniform progression in the concentration of Kepone in oysters with time (Figure 6). There was indication that an asymptotic level had been reached after two weeks. There also was a clear separation between the three lines which represented high, medium and low concentrations in sediments. A uniform progression was also evident in the second series of experiments although the absolute concentrations attained in oyster meats were lower than in the first series and there was no indication that an asymptotic level had been reached (Figure 7). In the third series there was neither a uniform progression nor suggestion of an asymptotic level.

The three sets of lines in Figures 6-8 did not appear to share a common pattern. However, they did show that the higher concentrations in oyster meats were associated with the higher concentrations in the sediments and vice versa. When the values for Kepone concentration in oyster meats in the three series of experiments (Tables 1-3) were grouped into three classes according to selected concentration ranges it was found that the values for Kepone in sediments also separated into three fairly distinguishable groups with different means. Eleven sediment values associated with concentrations in oyster meats between 0 and 0.10 ppm had a mean of 0.038 ppb (range: 0.020 - 0.098 ppb). Twelve values for concentration in sediment associated with concentrations in oyster meats between 0.101 and 0.199 had a mean of 0.058 ppb (range: 0.023 - 0.088 ppb). Five values for sediments associated with concentrations in oyster meats of 0.20 ppm or greater had a mean of 0.095 ppb (range: 0.070 - 0.113 ppb).

A plot of concentration of Kepone in oyster meats as a function of concentration in suspended sediments appears in Figure 9. Regression analysis showed a correlation between the two sets of data (correlation coefficient = 0.781).

Having obtained this correlation, the values for concentration in oyster meats were normalized on the basis of a constant, hourly concentration of Kepone in the sediments. The mean hourly concentration of Kepone in sediments for the whole duration of each experiment (approximately four weeks) was chosen as the normalization constant. The computed means appear in Table 4.

Plots of the normalized values for oyster meat concentrations appear in Figure 10 and 11. The marked dips in meat concentrations after two and three weeks of exposure during the third series of experiments have been eliminated in the normalized curves. The normalized curves suggest that an asymptotic level is reached after the first week of exposure in that series.

The curves for the first and second series were slightly altered by the conversion but the original trends shown were not appreciably changed. The curves for the first series still indicate an asymptotic plateau. Curves for the second series, on the other hand, still show a trend of increasing concentration in oyster meats with time. The high value seen for the third week in the borken line for the first period results from a relatively high value in the meats in the original data while the corresponding value in the suspended sediments was relatively low (medium concentration, Table 1).

There were significant differences in the temperatures at which the three series of experiments with oysters were conducted (Table 6). In the first series, York River water had to be heated to raise it to desirable levels. The minimum and maximum daily temperatures recorded near the source of our river water supply for each of the weekly periods included in the experiment were: 1st week, 3.2-7.6°C; 2nd week, 6.4-10.4°C; 3rd week, 9.0-12.8°C; and 4th week, 10.0-12.0°C. Water temperatures in the experimental trays ranged between 14.0 and 21.0°C during the four weeks included, with the average being between 17 and 18°C for each of the weekly periods.

The second and third series of experiments were conducted at ambient temperatures. These ranged between 18.3 and 25.7°C during the four weeks of the second series with an average for each week in the range of 20.9 to 23.5°C (Table 6). During the third series the overall range was 25.0 to 34.0°C with the weekly average ranging between 26.6 and 29.6°C.

During the first series of experiments, daily salinities ranged between 17.5 and 22.1‰ for the four weeks, and the weekly average ranged from 18.4 to 20.4‰ (Table 6). During the second series, the corresponding salinity ranges were 16.2 - 20.3‰ and 17.1-19.4‰. Likewise, the ranges of the corresponding averages for the third series were 20.2-23.6‰ and 20.6-23.1‰.

One of the experiments in the third series involved weekly analysis of Kepone concentration in the meats of oysters that had been held partially buried in an undisturbed bed of contaminated sediments. York River water flowing over the sediment bed was uncontaminated by Kepone. The concentration of Kepone in the sediments forming the bed averaged 1.77 ppm in two samples analyzed before the oysters were introduced (Table 7). A mixed sample from the same tray analyzed after the oysters were removed showed a concentration of 2.89 ppm. A sample collected from the top one centimeter layer of the tray after the oysters were removed had a Kepone concentration of 2.24 ppm.

After one week in the sediment bed the Kepone concentration in two samples of oysters averaged 0.037 ppm (Table 7). The concentration in oyster meats decreased gradually during the next three weeks below the detectability level of the analytical techniques, i.e., 0.02 ppm.

Mean sizes of oysters used in the three experiments appear in Table 9. They ranged between 7 and 8 cm in height during the first and third series of experiments and between 5 and 6 cm in the second series.

Oyster Biodeposits

Oysters concentrated Kepone in their biodeposits to levels thousands of times higher than those found in the suspended sediments (Table 8). The concentration factors for feces ranged from 11,000 to 55,000. In pseudofeces, the range was between 3,000 to 20,000. The concentration in feces was always higher than that in pseudofeces but the magnitude of the difference varied considerably between the paired sampled compared.

Concentration of Kepone in sediments that settled by gravity in the tray compartments was usually slightly higher than those in pseudofeces. However, it was also significantly lower than that in feces.

Rangia cuneata

Five experiments were conducted with the wedge clam *Rangia cuneata* during the second and third series of experiments. In four, animals were exposed to contaminated sediments in suspension and in one they were buried in a bed of contaminated sediments.

The results obtained for *Rangia* during the second series of experiments are almost identical to those obtained for oysters during the same series (Table 2, Figures 12 and 7). Most of the actual values found at any one weekly interval were close and the line trends are similar.

The data for Rangia in the thrid series of experiments were somewhat different from those for oysters (Table 3, Figures 13 and 8). Distribution of the weekly values for Rangia meats tended to remain at approximately the same level after the first week with a slight dip in the third week samples. The oyster data showed a greater vertical displacement of the weekly values. The data for both animals showed a fairly distinct separation between the lines for low and high Kepone concentrations in the sediments.

Rangia buried in undisturbed contaminated sediments accumulated Kepone to low levels (Table 7, Figure 13). After the first week high of 0.05 ppm there was a gradual decrease with time to 0.03 ppm after four weeks. Rangia receiving low concentrations of Kepone in suspension accumulated slightly more Kepone than those buried in the sediments even though the latter had a Kepone concentration several thousand times greater (2 ppm in the bed sediments vs. 0.02 to 0.06 ppb in the water column).

Water temperatures in the trays holding Rangia during the second series of experiments were slightly lower than during the third series (Table 6). The range during the second series was between 18 and 20°C and during the third series it was between 20 and 22°C. There was substantially no difference in water salinities during the two series.

Mean sizes and Rangia used in these experiments appear in Table 10. They ranged between 4 and 5 cm in height.

Macoma balthica

A single experiment was conducted with the clam Macoma balthica during the second series. The Macoma were held in the same tray with oysters receiving sediments in suspension at a high concentration of Kepone. However, they were placed in the tray one week later than the oysters and consequently, they remained in the tray one week after all the oysters had been removed.

The *Macoma* laid directly on the bottom of the tray and, being fairly small (average height was between 1.4 and 1.7 cm; Table 11) were in close contact with the contaminated sediments that settled on the tray bottom. Sediments settling to the bottom of the experimental trays were removed every two or three days.

The *Macoma* accumulated Kepone at the fastest rate of the three species studied to date. After three weeks the concentration was 0.33 ppm (Figure 14). During the fourth week there was a slight drop to 0.30 ppm.

Mean water temperatures in the trays holding *Macoma* ranged between 21 and 24°C during the four weekly periods (Table 6). Mean water salinities ranged between 17 and 20‰.

Mean sizes of *Macoma* used in these experiments appear in Table 11. They ranged around 1.5 cm in height.

Condition index. Measurements of the meat quality of samples of the experimental animals showed no significant differences between those analyzed at the start of the experiments and those analyzed after approximately four weeks in the experimental trays.

Discussion

The bivalves *Crassostrea virginica*, *Rangia cuneata* and *Macoma balthica* concentrated Kepone from suspended sediments by factors ranging between 1000 and 3000 over that in the water column.

There was little difference in the results obtained for Crassostrea and Rangia. Macoma, however, accumulated Kepone in greater concentrations than the other two species.

Crassostrea and Rangia showed similar trends in uptake of Kepone from suspension. This showed that the two species have similar feeding habits. As suspension feeders, they are reacting in a similar manner to the presence of the sediments in suspension. Such a similarity was reinforced by the experiments in which individuals of the two species were buried partly or fully in a bed of contaminated sediments. Neither one of the two species accumulated much Kepone under those circumstances. Water flow over the sediment beds was relatively slow and the water-sediment interface was not disturbed. Therefore, very little of the sediment was re-suspended. Concentrations in Rangia were slightly higher than those for oysters and if there is any significance to the difference it may be an indication that by being fully buried with its siphon close to the sediment surface, Rangia had access to sediments not available to oysters.

The data for oysters showed a strong correlation between the mean hourly concentration of Kepone in suspended sediments, computed for weekly intervals, and the mean concentration in oyster samples exposed to those sediments during the same weekly period. As illustrated in Figures 6-8, usually the Kepone in oyster meats decreased or increased from one week to the next following a decrease or increase in Kepone in the sediments during the intervening week. The validity of such a correlation is further reinforced by the similarity between the patterns of the curve for low and high sediment concentrations in each of the three series of experiments.

A weaker correlation (0.614) was also found in the data for *Rangia*. Further collection of data for *Macoma* will be necessary before it can be determined if the relationship holds for that species.

This correlation indicates that, at the temperatures included, oysters and possibly other bivalves such as *Rangia* and *Macoma* depurate themselves of Kepone continuously at the same time that they ingest and accumulate it. Therefore, in order for the Kepone level to remain at a high level, the Kepone concentration in suspension will also have to remain at a correspondingly high level.

Consequently, disturbance of river bottoms contaminated with Kepone by natural processes or other processes initiated by man, which would result in an increase in the suspended sediment load, appear to be capable of causing a sharp increase in the levels of Kepone in individuals of bivalve populations within reach of the increased load. On the other hand, it would appear that such an increase in Kepone in the affected animals would also decrease sharply once the disturbance is terminated.

It is difficult to evaluate with the data obtained to date the influence of temperature on the uptake and depuration of Kepone by oysters and *Rangia*. More data are required to establish that.

Further studies are planned to investigate this relationship between Kepone in sediments and in bivalves. The effect of concentrations in the sediments higher than those tested so far will be considered. The effect of higher water flows capable of causing suspension of surface sediments in a bed holding buried animals will also be studied. Experiments that include combinations of contamination and depuration of bivalves will also be conducted.

The levels of Kepone flowing over animals in experimental trays have been fairly low - never higher than 0.15 ppb in the water column - in the experiments conducted so far. This has been dictated by restrictions in the capability of our system and personnel to maintain larger quantities of sediments in stock suspensions and flowing over the animals around the clock for four weeks. Changes required to achieve higher sediment concentrations will be implemented in the forthcoming series of experiments.

The data indicate that a leveling in the concentration of Kepone in oysters and *Rangia* occurs after the first week of exposure. This was seen best in the curves obtained by normalization of the data using as a constant the mean hourly concentration of Kepone in the sediments for the duration of each experiment. Since no animal samples were analyzed for a period shorter than one week it is quite possible that the leveling may occur sooner than one week. Either way, this is another indication of the efficiency of these bivalves to depurate themselves of Kepone since it is evidently a balance between uptake and depuration that is responsible for the leveling off in the curves.

Analysis of oyster biodeposits indicated that Kepone is concentrated in feces to levels many thousand times higher than it is present in the water column. These observations re-emphasize the importance of the effect biodeposition can have on the physico-chemical characteristics of sediments. At the same time oysters accumulate Kepone in their tissues to levels up to 3000 times that in the water column, they are also re-depositing high concentrations of the chemical on the bottom. This redeposition is being done in the form of material less likely to be resuspended because of its nature as an aggregate.

Kepone concentration in oyster pseudofeces was not much different than that found in sediments that settled by gravity onto the tray bottom. Therefore, there appears to be no indication that pseudofeces contribute to the deposition of Kepone-rich sediments any more than natural sedimentation would. However, pseudofeces form an aggregate which like feces may also resist re-suspension to a greater extent than naturally-settling sediments.

There is no way to establish to what extent sediments settling by gravity in experimental trays are included in the samples of feces and pseudofeces collected. However, the concentrations recorded for feces are so much greater than in the natural sediments and the bulk of the feces was so obviously greater than the fine blanket of sediments on the bottom of the tray, that it can be safely infered that their contribution to the values recorded for feces are minimal.

Literature Cited

Haven, D. S. 1960. Seasonal cycle of condition index of oysters in the York and Rappahannock Rivers. Proc. Nat'l Shellfish Assoc. 54: 42-65.

Table 1. Concentration of Kepone in sediments and in the meats of oysters during successive exposure periods in first series of Kepone uptake experiments. 24 February - 27 March, 1977

Exposure Period	No. days	Sediments (ppb) Range	Sediments (ppb) Hourly Mean	Meats Mean (ppm)	Concentration Factor
Low Sediment Concentration					
1	6.9	0.014 - 0.039[1]	0.027	0.086	3185
2	14.8	0.014 - 0.066	0.037	0.125	3289
3	21.8	0.003 - 0.045	0.023	0.135	5625
4	29.2	0.015 - 0.046	0.033	0.113	3228
Medium Sediment Concentration					
1	6.9	0.027 - 0.083	0.057	0.130	2281
2	14.8	0.027 - 0.142	0.073	0.160	2078
3	21.8	0.006 - 0.091	0.045	0.185	3854
4	29.2	0.029 - 0.092	0.067	0.133	1900
High Sediment Concentration					
1	6.9	0.040 - 0.197	0.082	0.185	2256
2	14.8	0.054 - 0.197	0.104	0.250	2294
3	21.8	0.008 - 0.133	0.070	0.210	2838
4	29.2	0.044 - 0.137	0.098	0.257	2495

[1] Short period of time when no contaminated sediments were being added to the water flowing over the animals (i.e., sediment concentration = 0) are not included in range. However, they were used in computing the mean. This includes the final 8-9 hours when animals were allowed to flush out sediments in their digestive tract prior to removal for analysis.

Table 2. Concentration of Kepone in sediments and in animal meats during successive exposure periods in second series of Kepone uptake experiments. 13 May - 19 June, 1977.

Exposure Period	No. Days	Sediments (ppb)		Meats	Concentration Factor
		Range	Hourly mean	Mean (ppm)	
Low Concentration					
Oysters:					
1	7.3	0.024 - 0.078[1]	0.042	0.039	931
2	14.8	0.024 - 0.058	0.035	0.058	1667
3	22.0	0.017 - 0.040	0.026	0.064	2424
4	29.0	0.028 - 0.055	0.038	0.096	2526
Rangia:					
1	7.3	0.024 - 0.077	0.039	0.025	641
2	14.8	0.024 - 0.057	0.034	0.050	1453
3	22.0	0.016 - 0.039	0.025	0.048	1912
4	29.0	0.028 - 0.054	0.037	0.083	2237
High Concentration					
Oysters:					
1	7.2	0.054 - 0.178	0.098	0.09	905
2	14.7	0.058 - 0.139	0.086	0.16	1860
3	21.9	0.040 - 0.095	0.063	0.11	1732
4	28.9	0.068 - 0.132	0.093	0.23	2484
Rangia:					
1	7.2	0.057 - 0.188	0.104	0.05	521
2	14.7	0.061 - 0.147	0.091	0.14	1545
3	21.9	0.043 - 0.100	0.067	0.11	1644
4	28.9	0.071 - 0.140	0.098	0.22	2254
Macoma:					
1	7.5	0.058 - 0.139	0.086	0.13	1512
2	14.7	0.040 - 0.095	0.063	0.19	2992
3	21.7	0.068 - 0.132	0.093	0.33	3564
4	29.0	0.095 - 0.131	0.098	0.30	3067

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[1] Short periods of time when no contaminated sediments were being added to the water flowing over the animals (i.e., sediment concentration = 0 are not included in range. However, they were used in computing the mean. This includes the final 8-9 hours when arrivals were allowed to flush out sediments in their digestive tracts prior to removal for analysis.

Table 3. Concentration of Kepone in sediments and in the meats of oysters and *Rangia* during successive exposure periods in third series of Kepone uptake experiments. 8 July - 9 August, 1977.

Exposure Period	No. Days	Sediments (ppb) Range	Sediments (ppb) Hourly mean	Meats Mean (ppm)	Concentration Factor
Low Sediment Concentration					
Oysters:					
1	8.0	0.018 - 0.087[1]	0.047	0.113	2404
2	15.4	0.012 - 0.058	0.020	0.067	3350
3	23.4	0.007 - 0.041	0.020	0.049	2450
4	31.0	0.008 - 0.085	0.035	0.067	2030
Rangia:					
1	8.0	0.020 - 0.097	0.058	0.058	1000
2	15.4	0.014 - 0.066	0.026	0.063	2423
3	23.4	0.008 - 0.044	0.024	0.041	1708
4	31.0	0.008 - 0.082	0.041	0.068	1658
High Sediment Concentration					
Oysters:					
1	8.1	0.046 - 0.223	0.113	0.21	1858
2	15.5	0.031 - 0.096	0.043	0.10	2325
3	23.5	0.019 - 0.078	0.040	0.069	1725
4	31.0	0.019 - 0.195	0.088	0.16	1818
Rangia:					
1	8.1	0.058 - 0.284	0.153	0.12	784
2	15.5	0.039 - 0.121	0.065	0.12	1846
3	23.5	0.021 - 0.086	0.053	0.085	1604
4	31.0	0.023 - 0.230	0.126	0.125	992

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[1] Short periods of time when no contaminated sediments were being added to the water flowing over the animals (i.e., sediment concentration = 0) are not included in range. However, they were used in computing the mean. This includes the final 8-9 hours when animals were allowed to flush out sediments in their digestive tract prior to removal for analysis.

Table 4. Normalized values for Kepone concentration in oysters exposed in laboratory trays to suspensions of sediments contaminated with Kepone. Presented as a function of the mean hourly concentration in sediments for the duration of each experiment.

Exposure Period	Length of Exposure (days)	Mean hourly conc. Kepone for each period (ppb)	Mean hourly conc. Kepone for accumulated time periods (ppb)	Actual conc. Kepone in oyster meats[1] (ppm)	Normalized conc. Kepone in oyster meats[2] (ppm)
First series of experiments (24 Feb - 27 March 1977)					
1	6.9	0.027	0.027	0.087	0.097
2	14.8	0.037	0.032	0.125	0.101
3	21.8	0.023	0.029	0.136	0.177
4	29.2	0.033	0.030[3]	0.113	0.103
1	6.9	0.057	0.057	0.130	0.139
2	14.8	0.073	0.066	0.160	0.134
3	21.8	0.045	0.059	0.188	0.255
4	29.2	0.067	0.061[3]	0.133	0.121
1	6.9	0.082	0.082	0.185	0.203
2	14.8	0.104	0.094	0.250	0.216
3	21.8	0.070	0.085	0.209	0.269
4	29.2	0.098	0.090[3]	0.257	0.236
Second series of experiments (13 May - 11 June 1977)					
1	7.3	0.042	0.042	0.039	0.032
2	14.8	0.035	0.038	0.058	0.058
3	22.0	0.026	0.034	0.064	0.086
4	29.0	0.038	0.035[3]	0.096	0.088
1	7.2	0.098	0.098	0.090	0.078
2	14.7	0.086	0.092	0.160	0.158
3	21.9	0.063	0.083	0.110	0.148
4	28.9	0.093	0.085[3]	0.230	0.210

Table 4, (con'td)
Normalized values in oyster meats

Exposure Period	Length of Exposure (days)	Mean hourly conc. Kepone for each period (ppb)	Mean hourly conc. Kepone for accumulated time periods (ppb)	Actual conc. Kepone in oyster meats[1] (ppm)	Normalized conc. Kepone in oyster meats[2] (ppm)
Third series of experiments (8 July - 9 Aug. 1977)					
1	8.0	0.047	0.047	0.110	0.072
2	15.4	0.020	0.034	0.067	0.104
3	23.4	0.020	0.029	0.049	0.076
4	31.0	0.035	0.031[3]	0.067	0.059
1	8.1	0.113	0.113	0.210	0.133
2	15.5	0.043	0.080	0.100	0.167
3	23.5	0.040	0.066	0.069	0.124
4	31.0	0.088	0.072[3]	0.160	0.131

1 Determined analytically
2 Normalized value computed proportionally
3 Mean value reference used in computing normalized values in oysters

Table 5. Mean hourly concentration of Kepone in sediment suspensions flowing over *Rangia* and *Macoma* during the total duration of each period of exposure in experimental trays.

Species	Total duration of exposure (days)	Mean hourly concentration for each weekly period (ppb)	Mean hourly concentration for full period (ppb)
Rangia:	Second series of experiments (13 May - 11 June 1977)		
	Low sediment concentration		
	7.3	0.039	0.039
	14.8	0.034	0.037
	22.0	0.025	0.033
	29.0	0.037	0.034
	High sediment concentration		
	7.2	0.104	0.104
	14.7	0.091	0.097
	21.9	0.067	0.087
	28.9	0.098	0.090
	Third series of experiments (8 July - 9 August)		
	Low sediment concentration		
	8.0	0.058	0.058
	15.4	0.026	0.043
	23.4	0.024	0.036
	31.0	0.041	0.037
	High sediment concentration		
	8.1	0.153	0.153
	15.5	0.065	0.111
	23.5	0.053	0.091
	31.0	0.126	0.100

Table 5, Continued

Species	Total duration of exposure (days)	Mean hourly concentration for each weekly period (ppb)	Mean hourly concentration for full period (ppb)
Macoma:	Second series of experiments (8 July - 9 August 1977)		
	High sediment concentration		
	7.5	0.086	0.086
	14.7	0.063	0.075
	21.7	0.093	0.081
	29.0	0.098	0.085

Table 6. Range and mean of water temperature and salinity in trays holding animals during Kepone uptake experiments.

Weekly Period	Temperature (C)		Salinity (o/oo)	
	Range	Mean	Range	Mean
1st Series (Feb. 24 - March 27, 1977)				
Oysters:				
1st	14.0 - 20.8	17.2	19.3 - 22.1	20.4
2nd	15.0 - 21.0	17.7	19.1 - 20.6	20.2
3rd	16.1 - 20.8	18.5	19.1 - 20.1	19.7
4th	14.8 - 19.6	17.0	17.5 - 19.2	18.4
2nd Series (May 13 - June 19, 1977)				
Oysters:				
1st	18.3 - 25.0	20.9	17.5 - 19.2	18.3
2nd	21.3 - 25.0	22.4	16.2 - 17.9	17.1
3rd	22.3 - 25.7	23.5	17.5 - 19.5	18.3
4th	20.5 - 25.0	21.5	18.9 - 20.3	19.4
coma:				
1st	21.3 - 25.0	22.4	16.2 - 17.9	17.1
2nd	22.3 - 25.7	23.5	17.5 - 19.5	18.3
3rd	20.5 - 25.0	21.5	18.9 - 20.3	19.4
4th	20.7 - 25.9	23.7	19.9 - 20.0	19.9
Rangia:				
1st	16.6 - 21.2	18.6	0.5 - 7.3	5.5
2nd	18.7 - 20.8	19.5	5.0 - 6.4	5.4
3rd	19.0 - 22.3	20.4	1.3 - 7.9	5.1
4th	18.0 - 21.3	19.2	3.2 - 6.4	5.0
3rd Series (July 8 - August 9, 1977)				
Oysters:				
1st	26.9 - 34.0	29.6	20.2 - 20.8	20.6
2nd	26.8 - 32.0	29.3	20.9 - 22.1	21.6
3rd	25.0 - 30.0	26.6	21.9 - 22.9	22.5
4th	26.5 - 30.9	28.5	22.9 - 23.6	23.1
Rangia: 1st	20.5 - 24.3	22.9	2.8 - 8.7	5.9
2nd	20.4 - 24.9	22.5	3.9 - 8.8	6.1
3rd	19.0 - 22.0	20.3	4.2 - 6.0	5.4
4th	20.0 - 23.2	21.4	2.3 - 6.8	5.4

Table 7. Concentration of Kepone in the meats of oysters and Rangia held in control trays receiving no contaminated sediments and in test trays partially or fully buried in unsieved sediments contaminated with Kepone. July 8 - August 9, 1977. Means in parentheses.

Exposure Period	Cumulative No. Days	Kepone Conc. in Animals Buried in Sediments (ppm)	Kepone Conc. in Control Animals (ppm)
A. Oysters (partially buried in test trays):			
1	8.5	0.034 0.040 (0.037)	≤0.007
2	15.9	0.024	≤0.009
3	23.9	0.014 0.018 (0.016)	≤0.005
4	31.6	0.014 ≤0.009 (≤0.007)	≤0.004
Rangia (fully buried in mud)			
1	8.5	0.067 0.035 (0.051)	0.011
2	15.9	0.053 0.039 (0.046)	≤0.006
3	23.9	0.029 0.038 (0.033)	≤0.003
4	31.6	0.034 0.031 (0.032)	≤0.007

Table 7 (Continued)

B. Concentration of Kepone (in ppm) in unsieved sediments used in test trays in which animals were fully or partially buried.

1. Mixed samples at start of experiment: (Same sediments used in both trays)	0.71 2.83 (1.77)
2. Fractionated and mixed samples at end of experiment:	
a. Mixed sample from oyster trays	2.89
b. Sample from top 1-cm layer in oyster tray	2.24
c. Mixed sample from *Rangia* tray	2.12
d. Sample from top 1-cm layer in *Rangia* tray	0.64

Table 8

Concentration of Kepone in oyster biodeposits and in sediments settling out by gravity in same tray holding the oysters.

Log Book and Page No.	Date Collected 1977	No. Days of Accumulation	Mean Hourly Concentration in Suspended Sediments (ppb)	Feces (ppm)	Feces Concentration Factor	Pseudo-feces (ppm)	Pseudofeces Concentration Factor	Gravity-Settling Sediments (ppm)	Gravity-Settling Sediments Concentration Factor
[illegible]	22 March	6	--	1.59		0.018			
[illegible]-23	4 April	5	--	0.33		0.13		0.13	
[illegible]-32	15 July	7	0.047	Lost		0.36	(7,659)	0.51	(10,851)
	15 July	7	0.113	1.30	(11,504)	0.34	(3,008)	0.41	(3,628)
[illegible]	23 July	7	0.025	0.80	(32,000)	0.50	(20,000)	0.52	(20,800)
	23 July	7	0.043	0.76	(17,674)	0.60	(13,953)	0.65	(15,116)
[illegible]	31 July	6	0.020	0.79	(39,500)	0.33	(16,500)	0.47	(23,500)
	31 July	6	0.040	1.23	(30,750)	0.29	(7,250)	0.48	(12,000)
[illegible]	9 August	7	0.033	1.83	(55,454)	0.57	(17,272)	0.23	(6,969)
	9 August	7	0.088	1.83	(20,795)	0.30	(3,409)	0.37	(4,204)

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Table 9. Mean height (in cm) of oysters in different samples analyzed for Kepone during uptake experiments. Number of animals in each sample appears in parentheses.

Exposure Period	Low Kepone conc. in sediments	Medium Kepone conc. in sediments	High Kepone conc. in sediments	Animals Buried in mud	Control Animals
First series of experiments (24 Feb - 27 March 1977)					
1	(4) 6.7 (3) 7.8	(4) 7.2 (3) 7.1	(4) 6.1 (3) 7.0		(4) 7.8 (3) 7.3
2	(4) 7.7 (3) 7.4	(4) 7.6 (3) 7.5	(4) 7.1 (3) 7.0		(4) 7.1 (3) 7.7
3	(4) 7.2 (3) 7.0	(4) 7.3 (3) 7.1	(4) 6.7 (3) 6.6		(4) 7.6 (3) 7.3
4	(4) 7.1 (3) 7.2 (4) 7.8	(4) 6.1 (4) 7.3 (5) 7.3	(4) 7.8 (4) 7.8 (4) 7.4		(4) 7.8 (4) 8.4 (3) 7.5
Second series of experiments (13 May - 19 June 1977)					
1	(8) 5.8		(8) 5.7		
2	(4) 6.0 (4) 5.4		(4) 5.6 (4) 4.3		(2) 7.9 (4) 6.0
3	(3) 6.9 (5) 5.4		(3) 6.4 (5) 4.8		(4) 6.3 (4) 4.9
4	(4) 5.7 (4) 5.1 (5) 5.6		(6) 5.1 (5) 5.5		(3) 6.6 (5) 5.4
Third Series of experiments (8 July - 9 Aug., 1977)					
1	(3) 8.1 (2) 7.7		(3) 7.2 (2) 7.9	(3) 6.6 (3) 7.2	(4) 7.5
2	(3) 7.9 (3) 7.6		(3) 7.6 (3) 7.6	(3) 6.1 (3) 7.4	(4) 7.7

Table 9, Cont'd)

posure Period	Low Kepone conc. in sediments	Medium Kepone conc. in sediments	High Kepone conc. in sediments	Animals Buried in mud	Control Animals
3	(3) 7.6 (3) 7.5		(3) 7.5 (3) 7.0	(3) 6.9 (3) 7.3	(4) 7.7
4	(4) 7.1 (4) 6.8		(3) 7.7 (4) 6.3	(4) 7.0 (3) 7.4	(3) 7.7 (3) 8.2

Table 10. Mean height (in cm) of *Rangia* in different samples analyzed for Kepone during uptake experiments. Number of animals in each sample appears in parentheses.

Exposure period	Low Kepone conc. in sediments	High Kepone conc. in sediments	Animals buried in mud	Control Animals
Second series of experiments (13 May - 19 June 1977)				
1	(8) 4.9	(8) 4.6		(8) 4.8
2	(4) 4.9 (4) 4.9	(4) 4.8 (4) 4.8		(4) 5.0 (4) 4.8
3	(4) 4.7 (4) 4.7	(4) 4.7 (4) 4.8		(4) 4.7 (4) 4.8
4	(8) 4.6 (8) 4.7 (8) 4.8	(8) 4.7 (8) 4.7 (8) 4.6		(8) 4.5 (7) 4.7
Third series of experiments (8 July - 9 Aug. 1977)				
1	(4) 5.01	(2) 5.31 (3) 4.90	(3) 4.85 (3) 5.04	(5) 5.20
2	(4) 4.99 (4) 4.49	(4) 4.88 (4) 4.92	(4) 4.92 (3) 5.02	(6) 4.74
3	(4) 5.00 (4) 5.15	(5) 5.02 (4) 4.89	(4) 5.12 (4) 4.96	(6) 4.98
4	(4) 5.03 (5) 4.73	(5) 4.83 (6) 4.79	(5) 5.00 (5) 5.24	(5) 4.88 (5) 4.75

Table 11. Mean height (in cm) of *Macoma* in different samples analyzed for Kepone during uptake experiments. Number of animals in each sample appears in parentheses.

Exposure Period	High Kepone conc. in sediments	Control Animals
Second series of experiments (13 May - 19 June 1977)		
1	(15) 1.7	(10) 1.6
2	(12) 1.6	(10) 1.6
3	(12) 1.5	(11) 1.6
4	(10) 1.4	(7) 1.6

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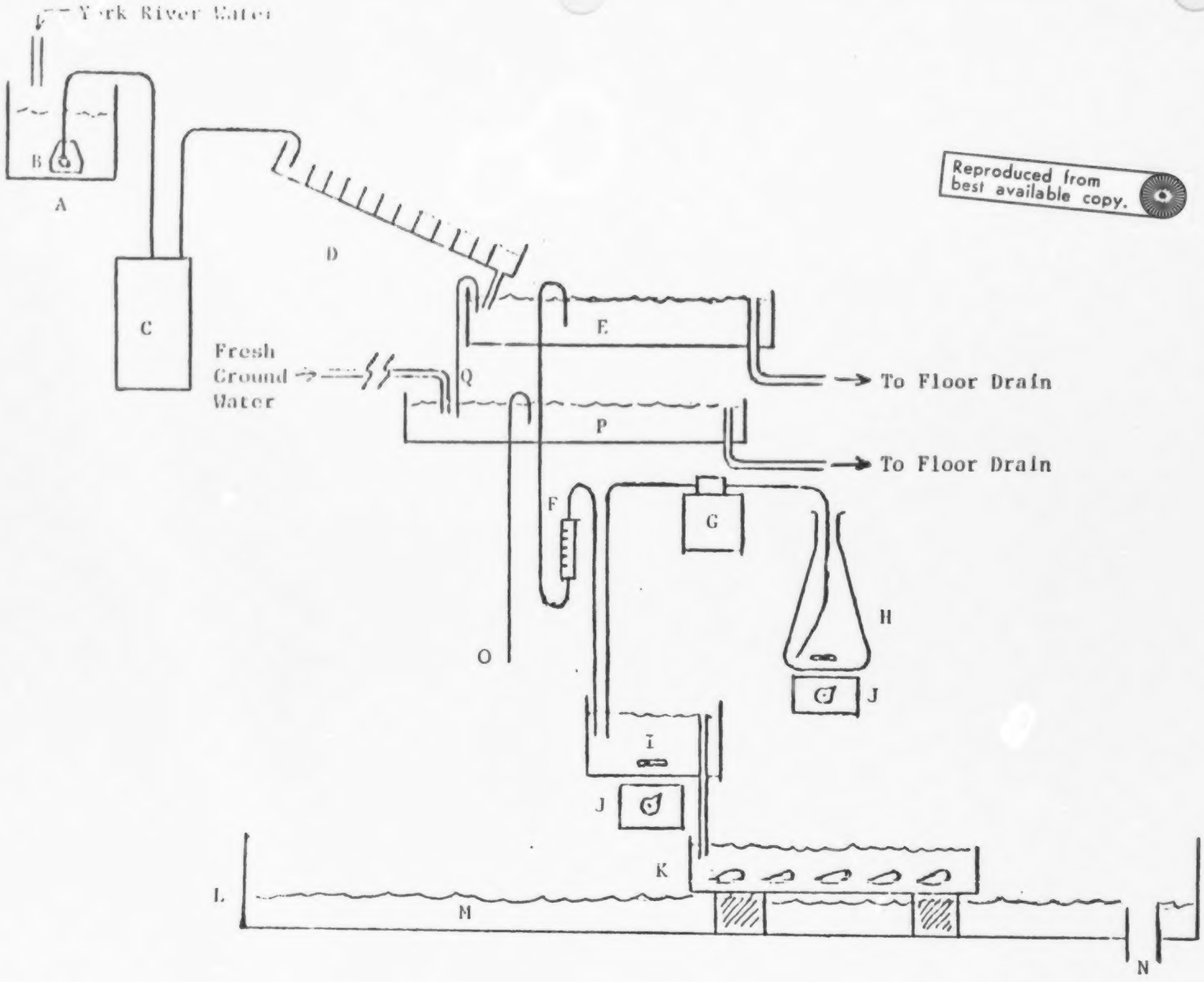
York River Water
A
B
C
D
Fresh Ground Water
Q
E
P
To Floor Drain
To Floor Drain
F
G
H
J
O
I
J
K
L
M
N

Figure 1. Setup of apparatus used in uptake experiments with bivalve molluscs in three series of experiments. Identification of individual components appears on next page.

Key to identification of components in Figure 1

A. Constantly-overflowing box providing York River water supply to system.

B. Submersible pump.

C. Heat exchanger system.

D. Cascading trough used to allow escape of gases coming out of suspension as result of river water being heated up.

E. Constantly-overflowing overhead trough from which water for experimental trays was siphoned.

F. Flow meter.

G. Peristaltic pump used to meter out sediment suspension.

H. Flask holding sediment suspension.

I. Mixing chamber receiving simultaneously York River water and sediment suspension.

J. Magnetic stirrer.

K. Experimental tray holding oysters.

L. Wet table holding experimental trays.

M. Drain pipe maintained a water level of about one-inch on wet table. This served as first component of a series of sediment trays.

N. Water from wet table overflowed into a series of three other sediment traps.

O. Siphon to mixing chamber of *Rangia* trays.

P. Constantly-flowing overhead trough from which water of low salintiy for experimental trays was siphoned.

Q. Siphon used to add river water from Trough E to fresh water in Tray P.

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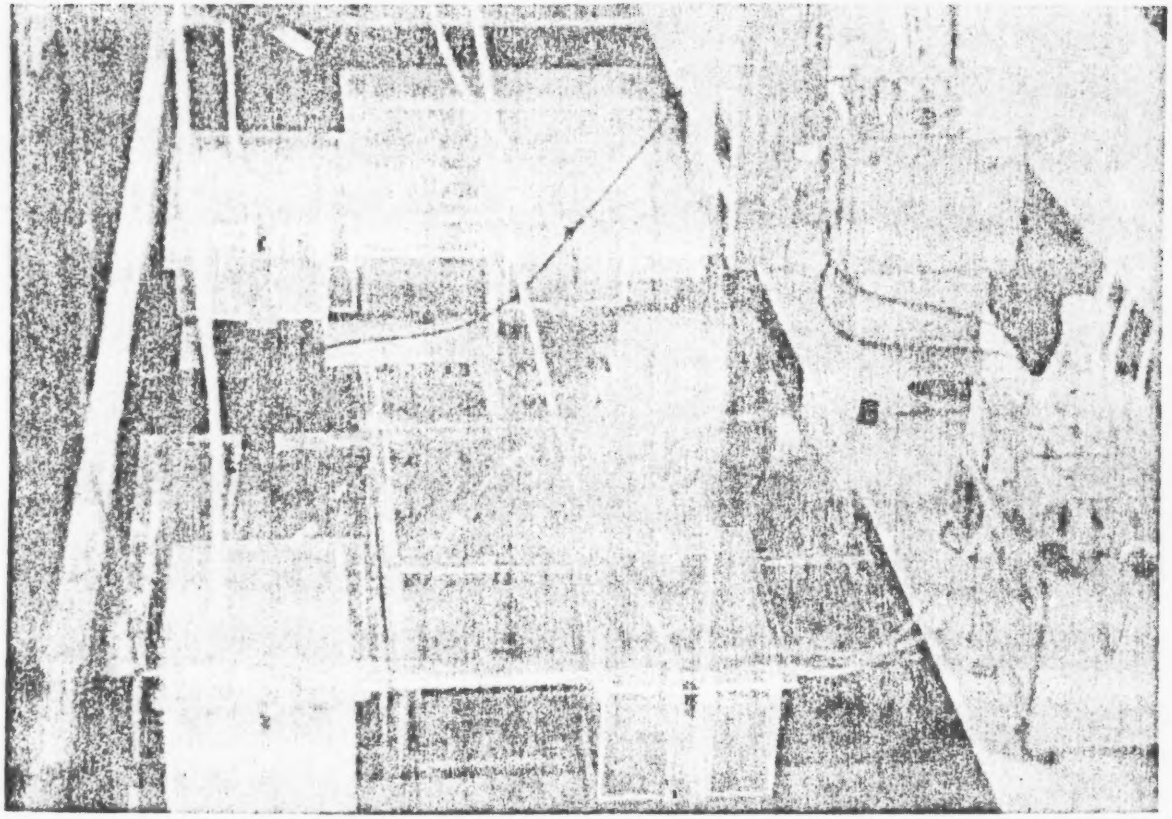

Figure 2. Arrangement of trays, mixing chambers and peristaltic pumps in third series of experiments in which animals received contaminated sediments in suspension. Oysters in large trays and *Rangia* in small ones.

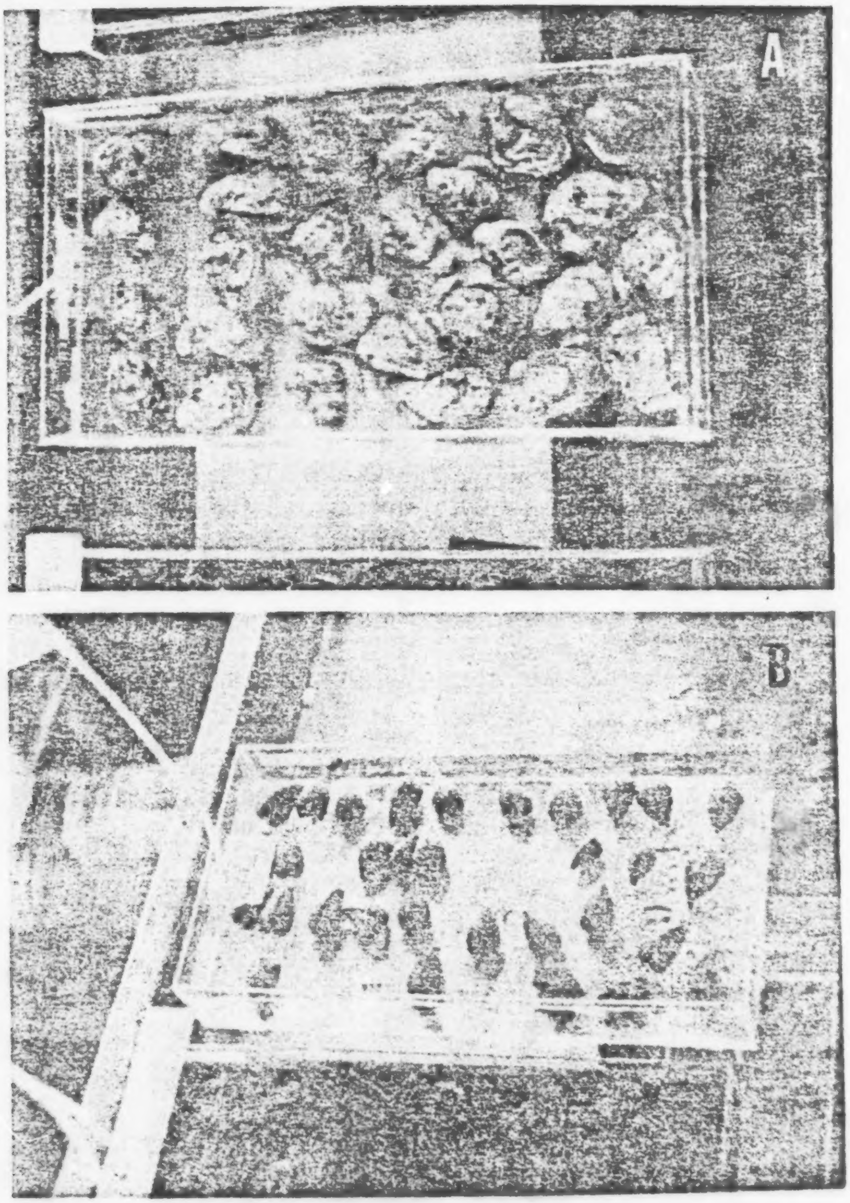

Figure 3. Control oysters (A) and Rangia (B) in small trays at start of third series of experiments.

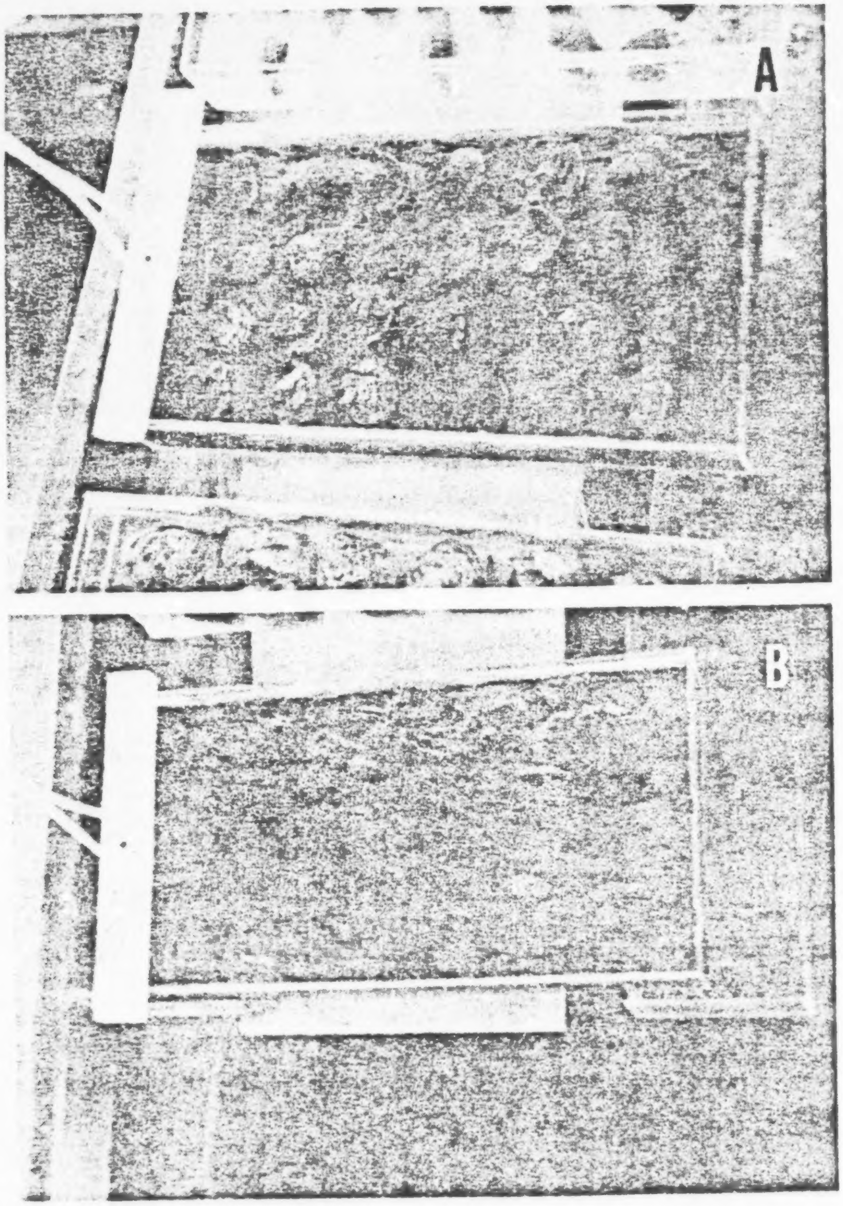

Figure 4. Oysters (A) and Rangia (B) partially buried in bed of sediments contaminated with Kepone at start of third series of experiments. Subsequently Rangia buried themselves fully.

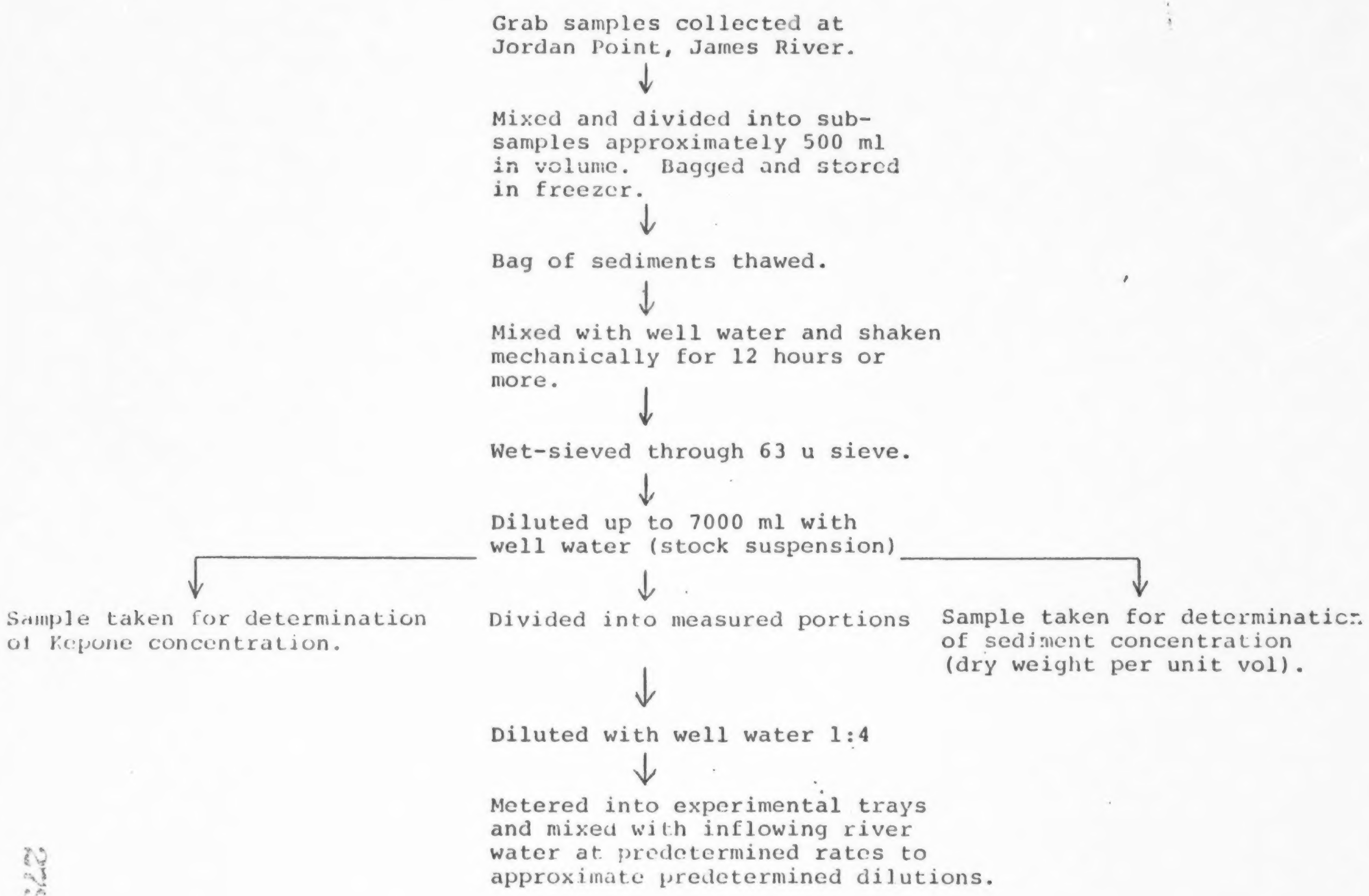
Grab samples collected at Jordan Point, James River.
Mixed and divided into sub-samples approximately 500 ml in volume. Bagged and stored in freezer.
Bag of sediments thawed.
Mixed with well water and shaken mechanically for 12 hours or more.
Wet-sieved through 63 u sieve.
Diluted up to 7000 ml with well water (stock suspension)
Sample taken for determination of Kepone concentration.
Divided into measured portions
Sample taken for determination of sediment concentration (dry weight per unit vol).
Diluted with well water 1:4
Metered into experimental trays and mixed with inflowing river water at predetermined rates to approximate predetermined dilutions.

Figure 5. Flow chart showing steps taken in preparation of sediments contaminated with Kepone for introduction into trays holding experimental animals.

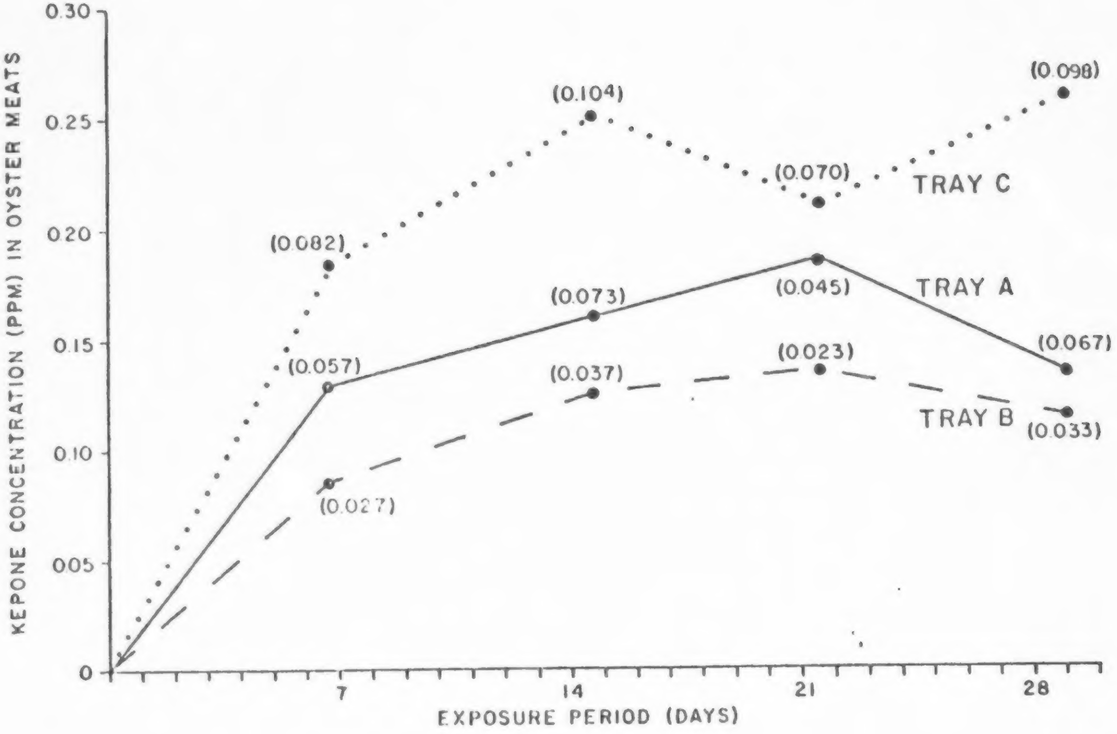

Figure 6. Mean concentration of Kepone in meats of oysters exposed to contaminated sediments in suspension. First series of experiments, 24 Feb.-27 March 1977. Figures in parentheses are mean hourly concentration of Kepone in sediments for weekly period ending at that point.

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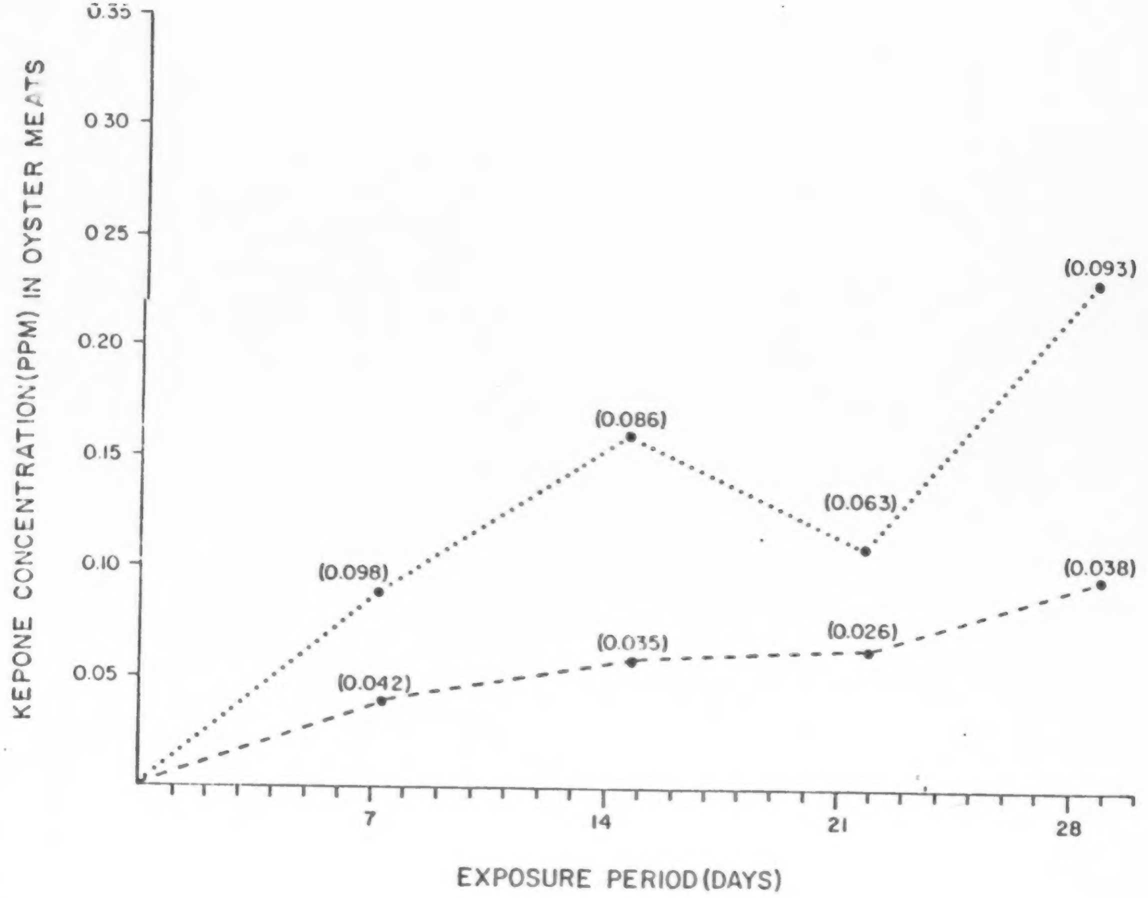

Figure 7. Mean concentration of Kepone in meats of oysters exposed to contaminated sediments in suspension. Second series of experiments, 13 May-11 June 1977. Figures in parentheses are mean hourly concentration of Kepone in sediments for weekly period ending at that point.

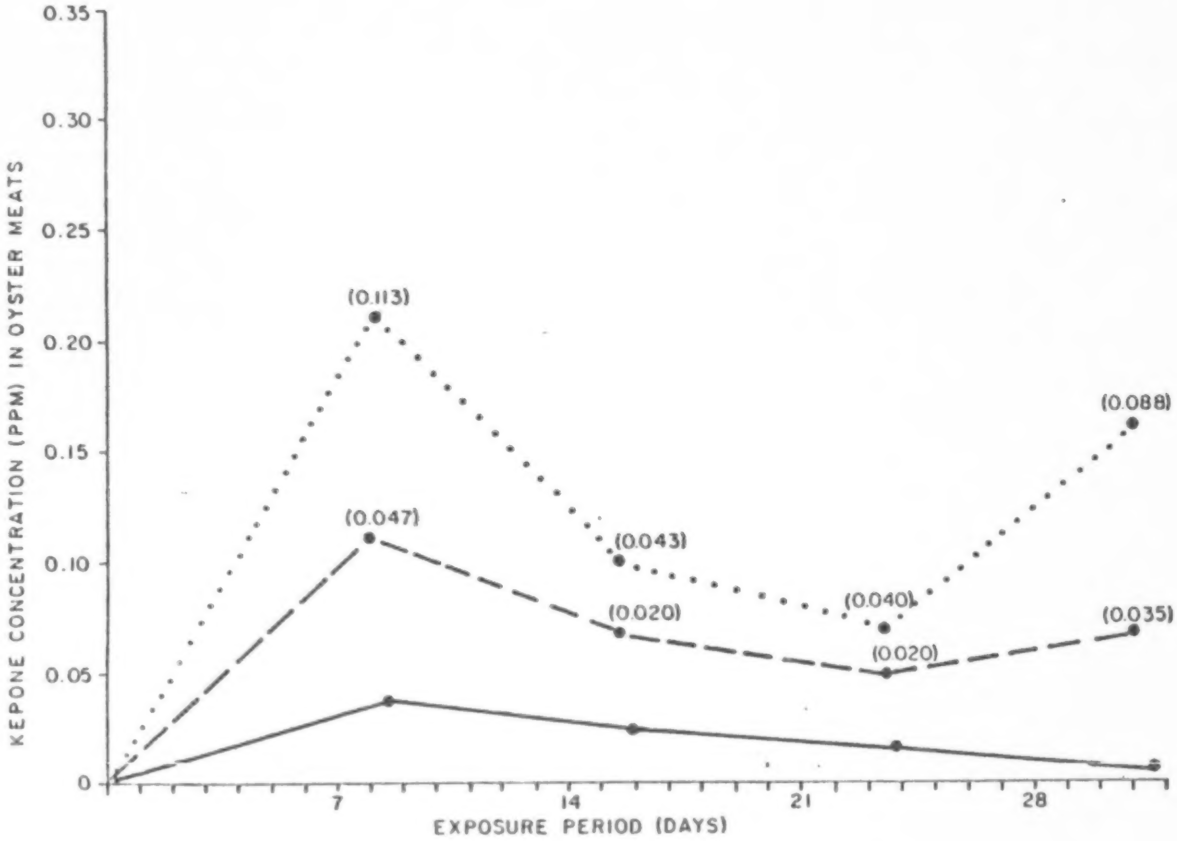

Figure 8. Mean concentration of Kepone in meats of oysters exposed to contaminated sediments in suspension (broken lines) or partially buried in bed of contaminated sediments (solid line). Third series of experiments, 8 July-9 August 1977. Figures in parentheses are mean hourly concentration of Kepone in sediments for weekly period ending at that

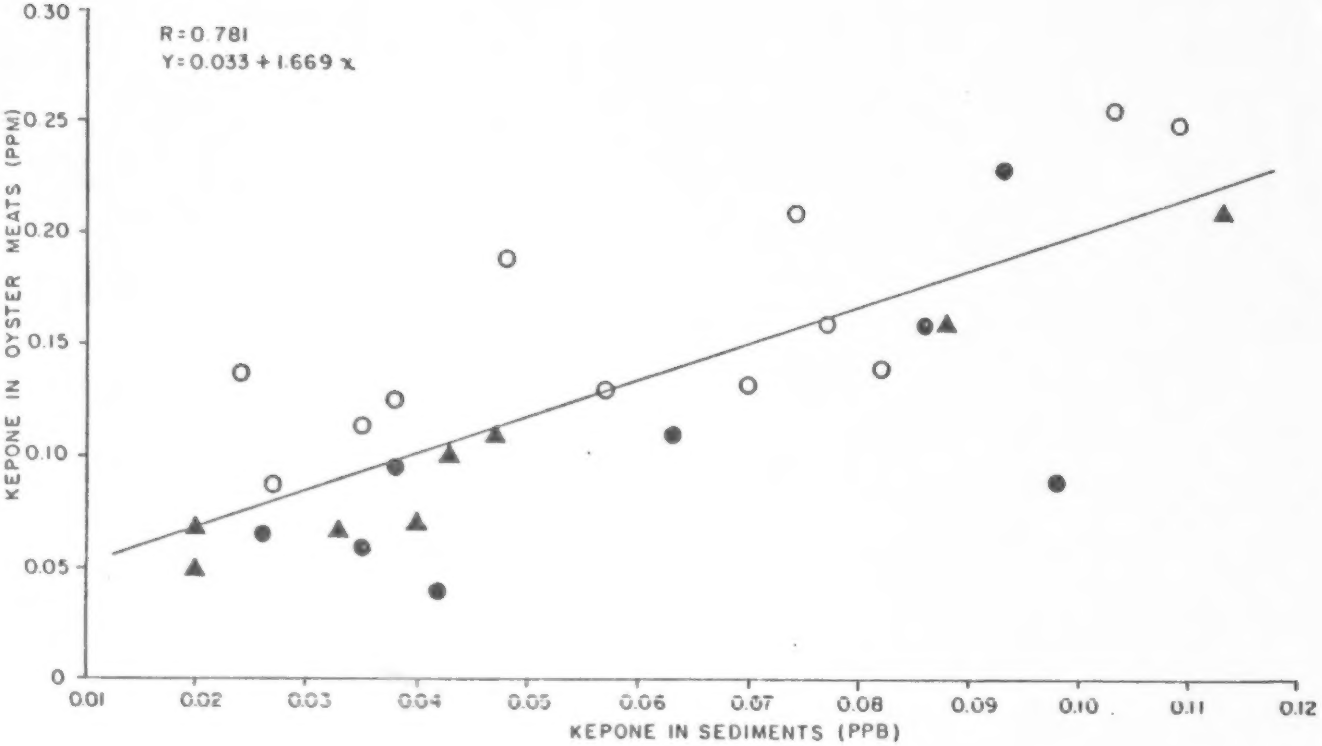

Figure 9. Regression of concentration of Kepone in oyster meats on mean hourly concentration of Kepone in suspended sediments for weekly periods in three series of experiments. Open circles: first series, closed circles: second series, triangles: third series.

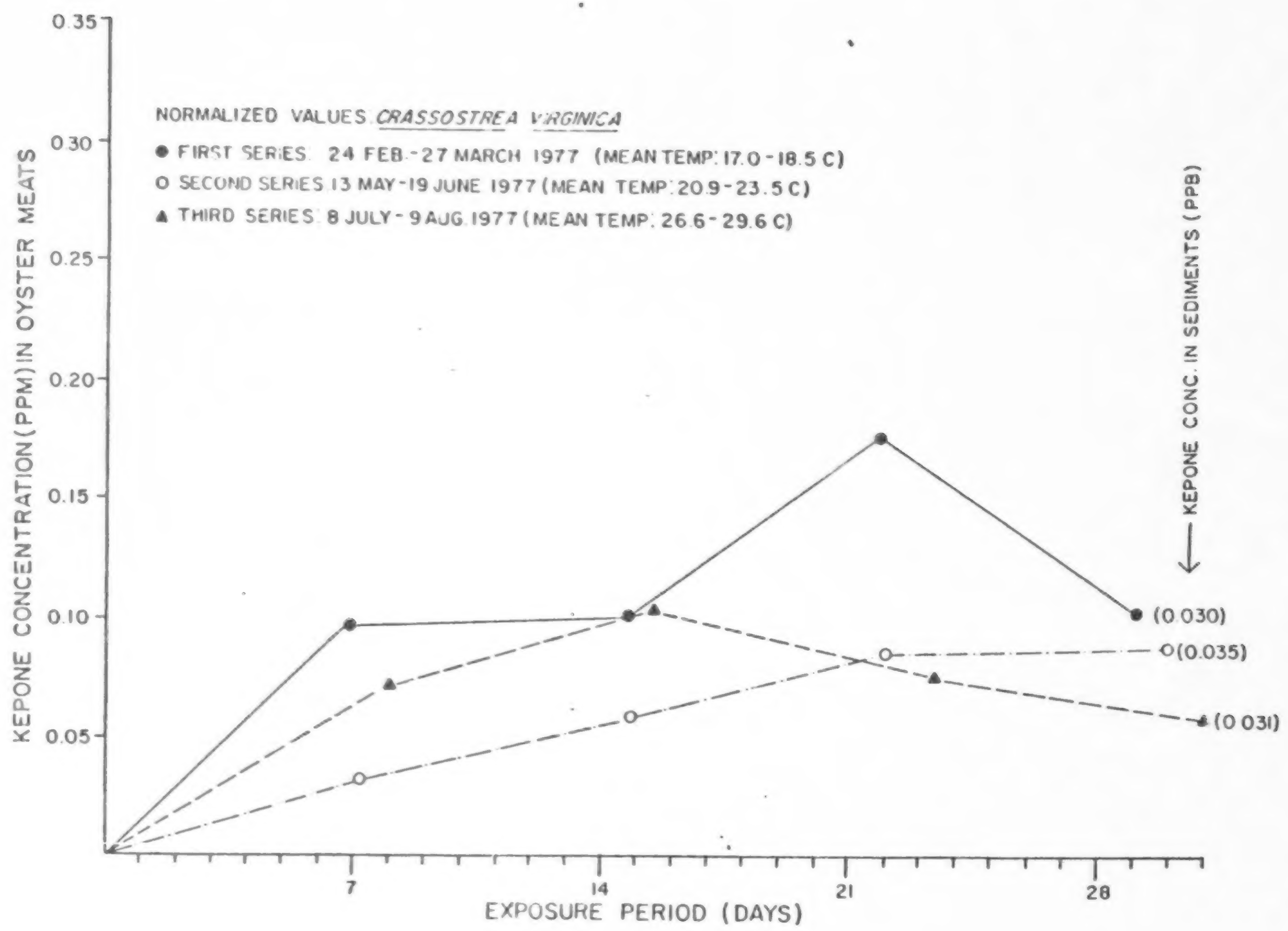

Figure 10. Mean concentration of Kepone in meats of oysters exposed to contaminated sediments in suspension. Normalized a constant hourly concentration for the four-week period in each series. Mean given in parentheses.

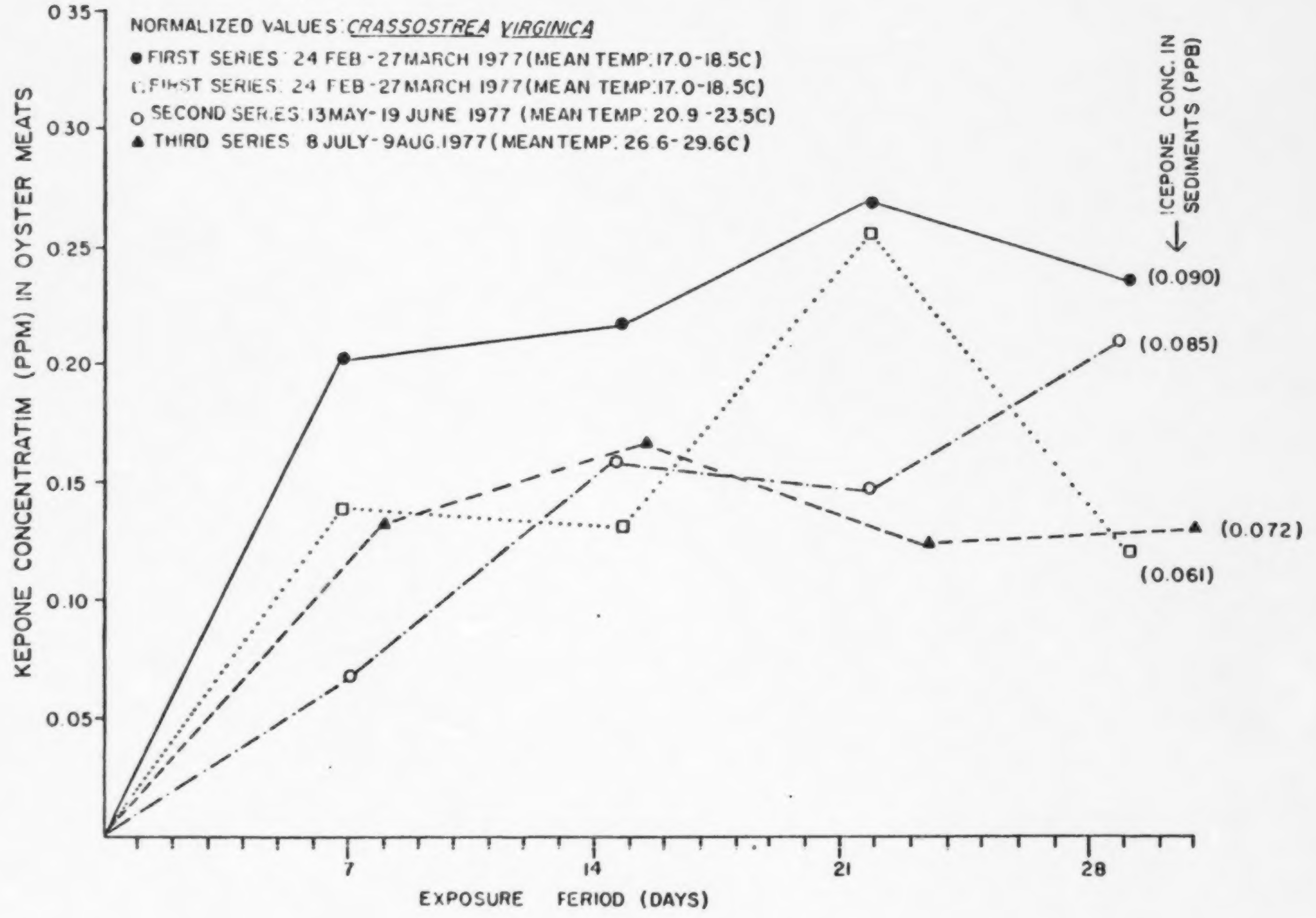

Figure 11. Mean concentration of Kepone in meats of oysters exposed to contaminated sediments in suspension. Normalized to a constant hourly concentration, the mean for the four-week period in each series. Mean given in parentheses.

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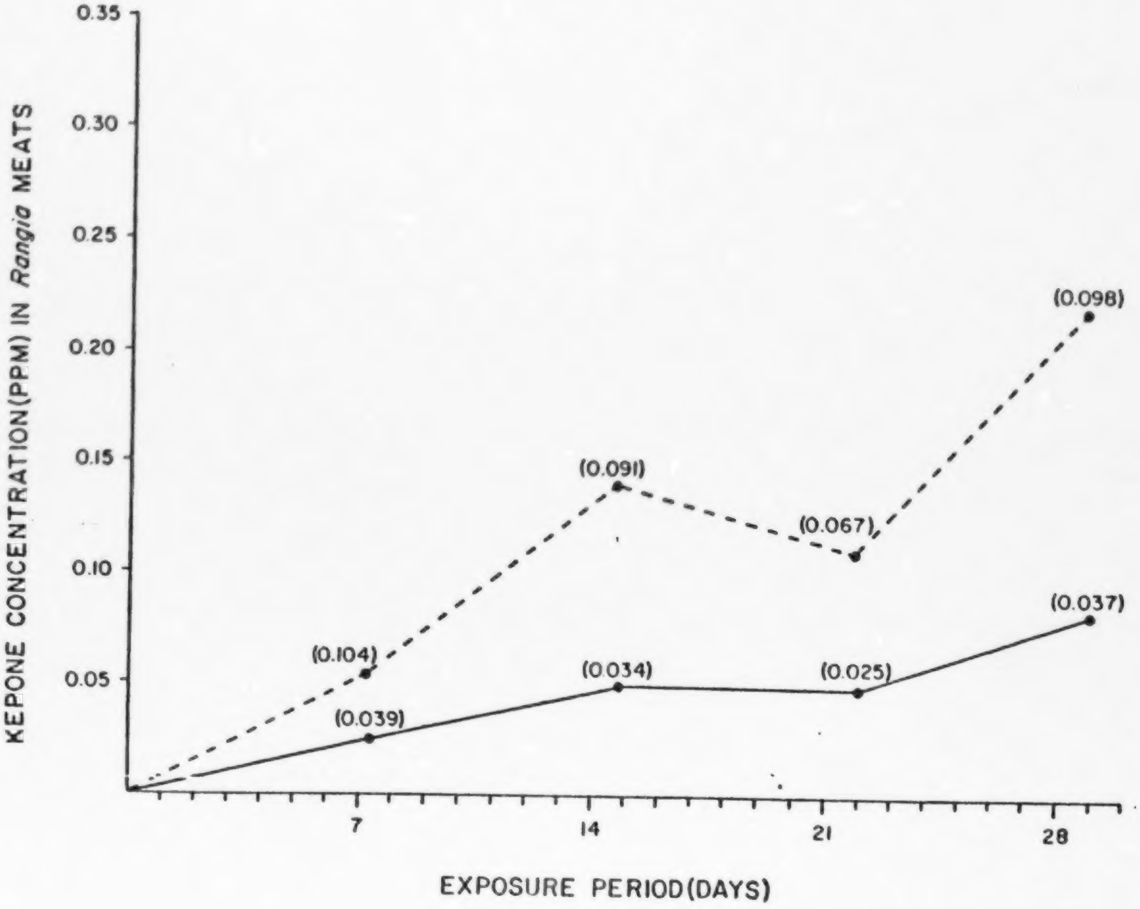

Figure 12. Mean concentration of Kepone in meats of Rangia cuneata exposed to contaminated sediments in suspension. Second series of experiments, 13 May-11 June 1977. Figures in parentheses are mean hourly concentration of Kepone in sediments for weekly period ending at that point.

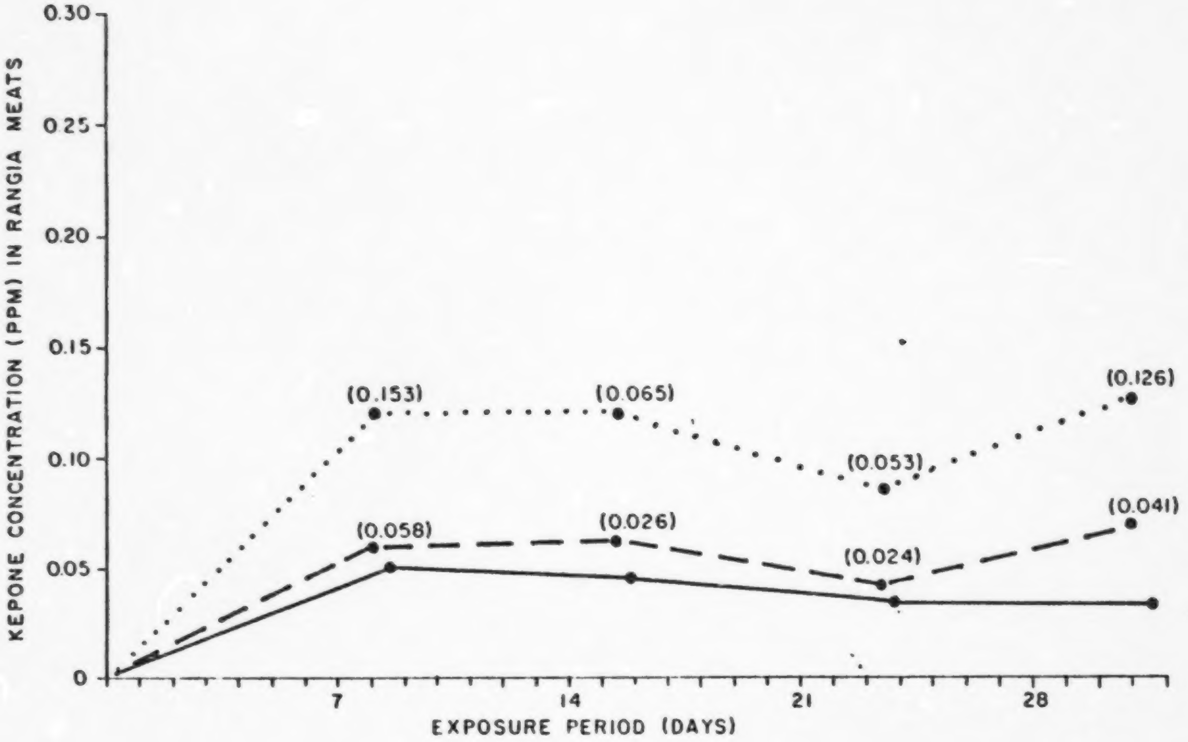

Figure 13. Mean concentration of Kepone in meats of *Rangia cuneata* exposed to contaminated sediments in suspension (broken lines) or buried in bed of contaminated sediments (solid line). Third series of experiments, 8July-9 August 1977. Figures in parentheses are mean hourly concentration of Kepone in sediments for weekly periods ending at that point.

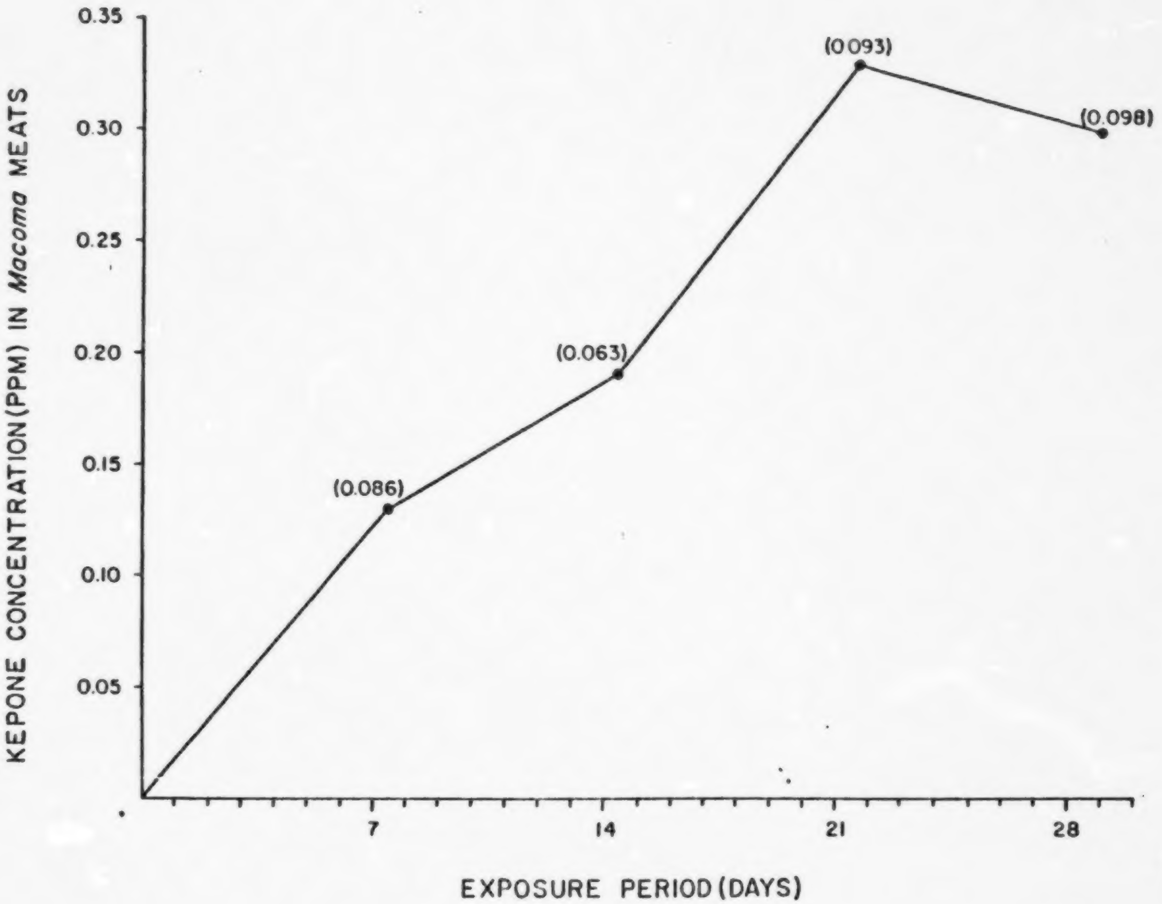

Figure 14. Mean concentration of Kepone in meats of *Macoma balthica* exposed to contaminated sediments in suspension. Second series of experiments, 20 May-19 June 1977. Figures in parentheses are mean hourly concentration of Kepone in sediments for weekly periods ending at that point.

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EPA James River Kepone Hydrographical Survey Study
Progress Report (Nov. 1, 1977)

I. Hydrographical Survey (Aug., 1977)

Four transects were occupied for the field study with three stations included in each transect. The middle (primary) station or primary station, measured top, middle and bottom depth, while the two side channel stations measured top and bottom depths. (figures of the transect positions are included within).

The following is a compilation of information concerning each station.

James River Station, River mile 46.51, sampled from 8/26/77 at 1500 to 8/28/77 at 1800.

Station 46.51A - total depth 17 feet

Current meter depth off the bottom: 2 feet and 7.5 feet

Current meter time in: 8/23/77 at 1015

Current meter time out: 8/29/77 at 1935

Samples taken at mid depth, included all parameters except kepone

Station 46.51 B - total depth 19.5 feet

Current meter depth off the bottom: 3 feet and 10.5 feet

Current meter time in: 8/23/77 at 1050

Current meter time out: 8/29/77 at 1925

Samples taken at top, mid and bottom depths, included all parameters

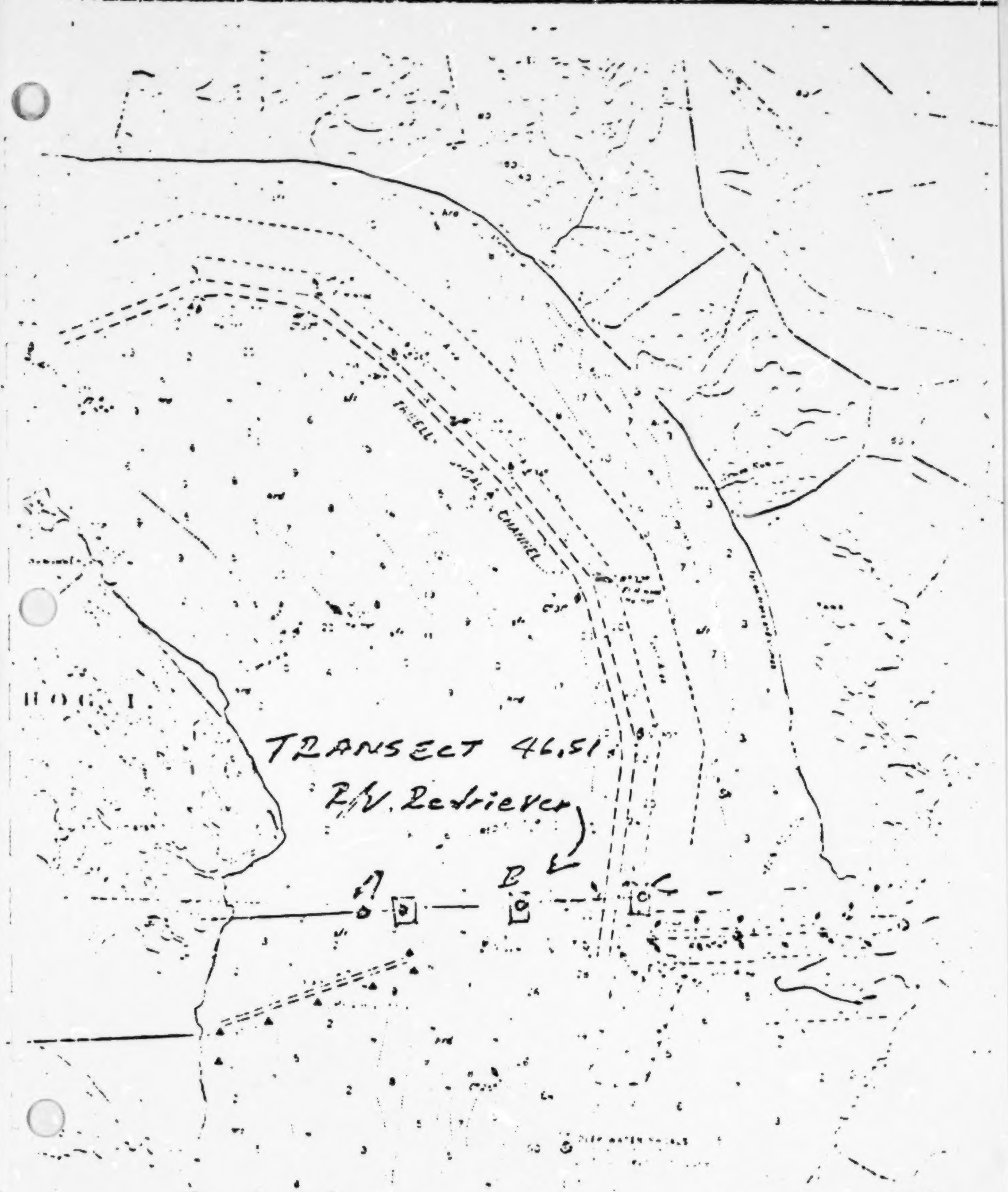
Station 46.51 C - total depth 23 feet

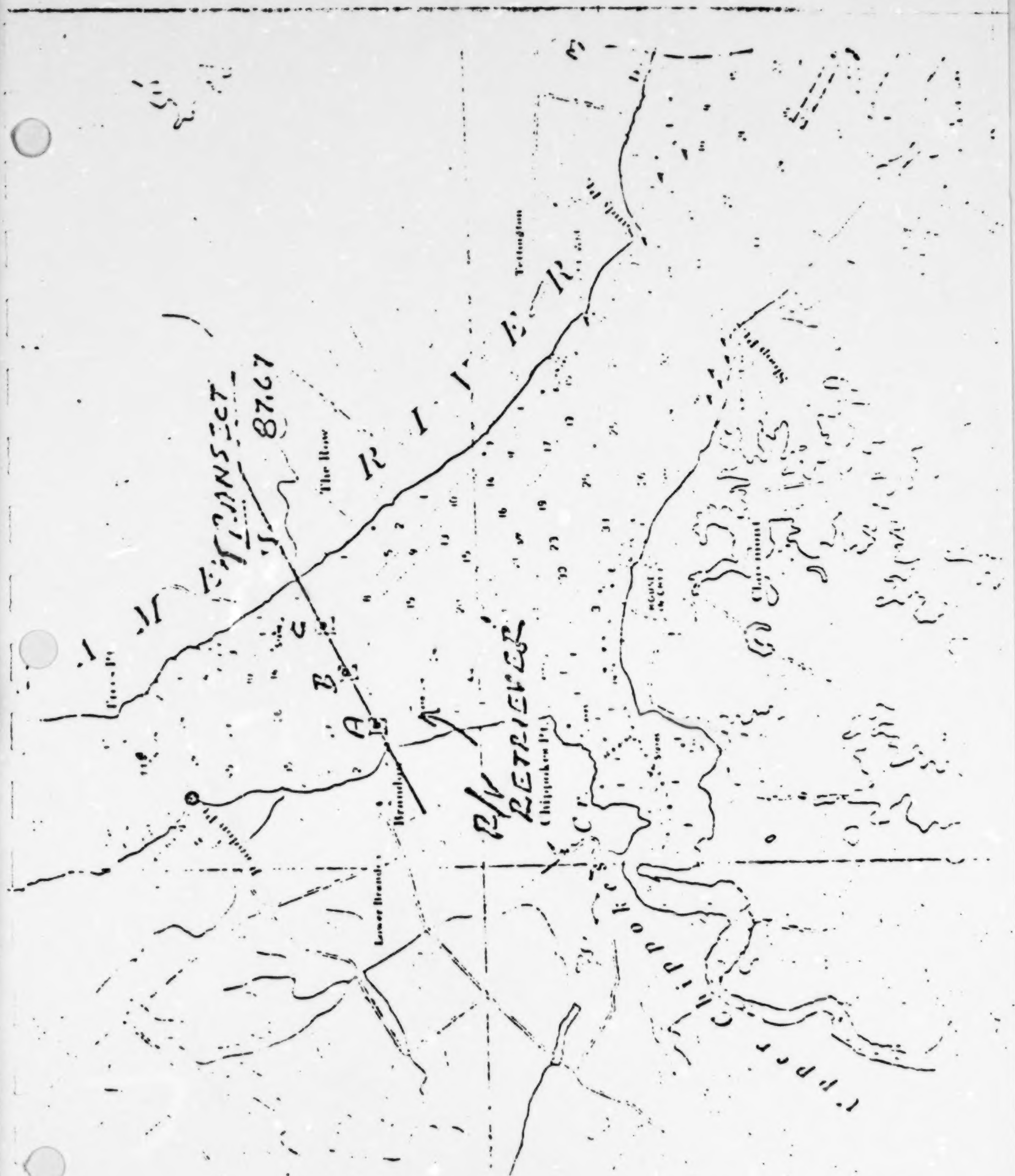
Current meter depth off the bottom: 2 feet, 6.5 feet and 12.5 feet

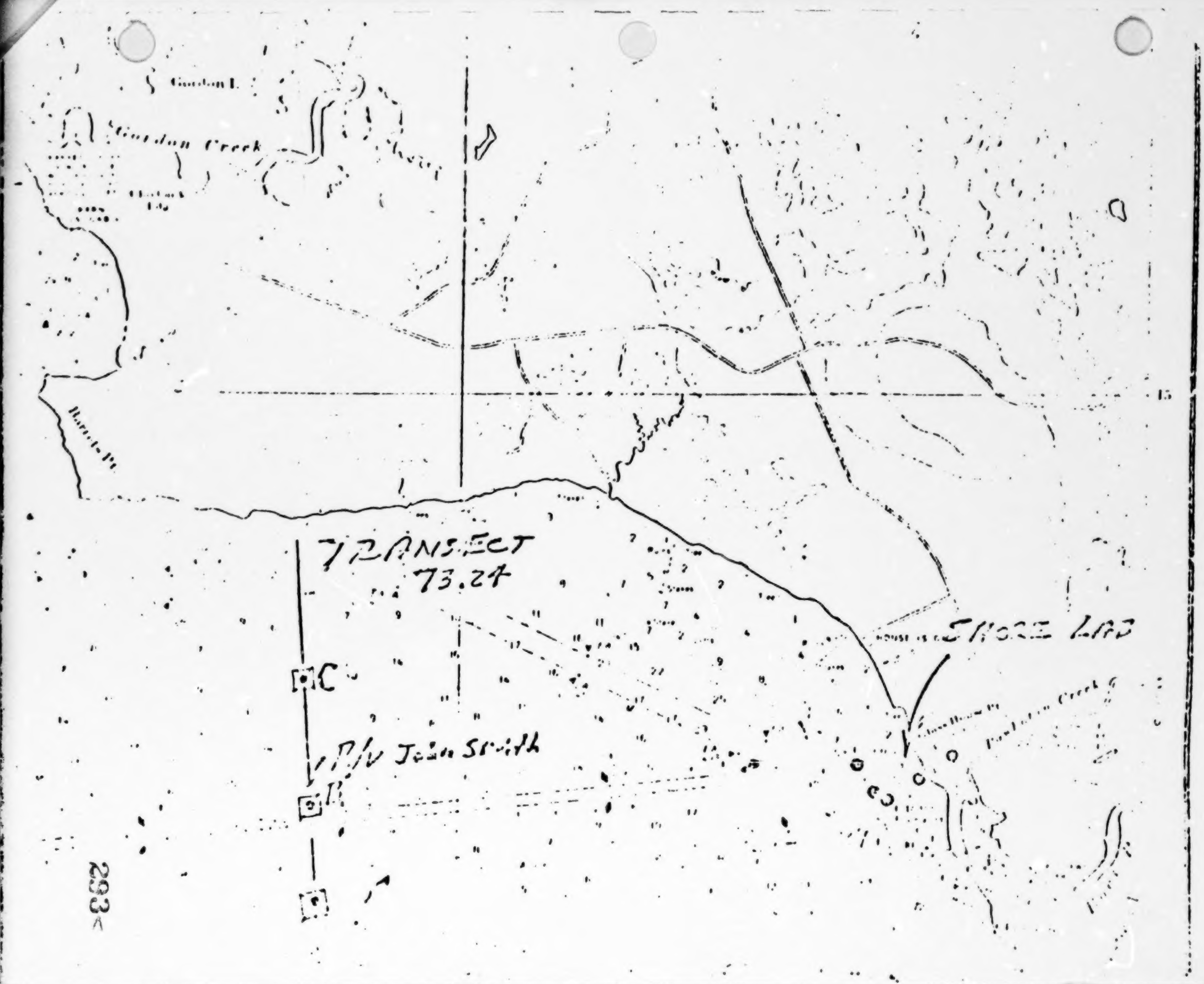
Current meter time in: 8/23/77 at 0940

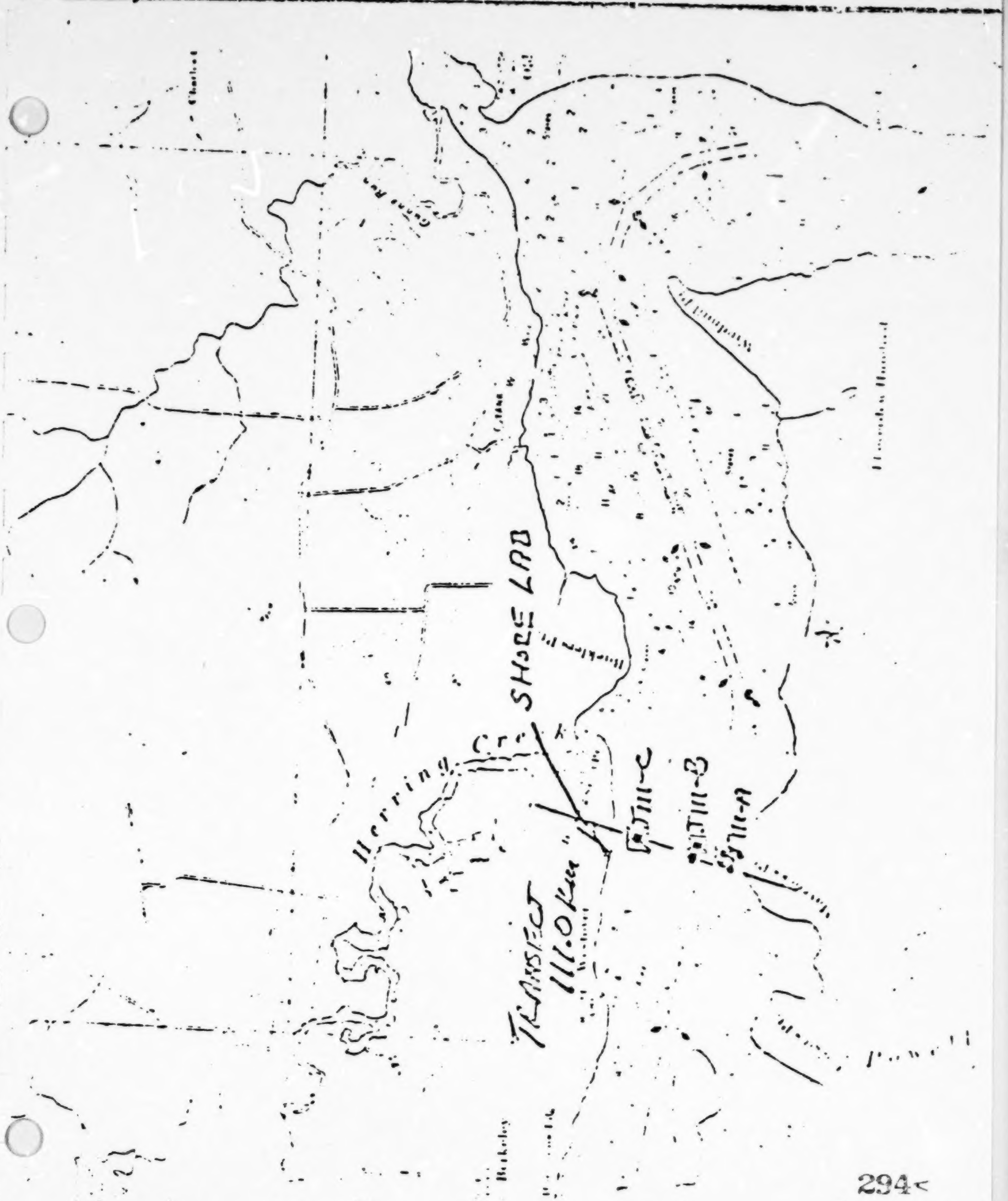
Current meter time out: 8/29/77 at 1915

Samples taken at mid depth, included all parameters, except kepone


TRANSECT 46,51.
R/V. Retriever
A
B
C
HOG I.
CHANNEL


JAMES RIVER
TRANSECT I
87.67
A
B
C
The Row
Brandon
Lower Brandon
R/V RETRIEVER
Chippokes Pt.
Upper Chippokes Ck.


Gordon I.
Gordon Creek
Barrets Pt.
15
TRANSECT
73.24
SHORE LAB
C
R/V John Smith
B


SHORE LAB
Herring Creek
TRANSECT III.0 Km
TIII-C
TIII-B
TIII-A
Powell

Station 73.24 sampled from 0800 8/27/77 to 8/29/77 1100

Station 73.24 A - total depth 15.5 feet

Current meter depth off the bottom:
6 feet
Current meter time in:
8/23/77 at 1550
Current meter time out:
8/29/77 at 1523
Samples taken at mid depth, included all parameters except kepone

Station 73.24 B - total depth 21.5 feet

Current meter depth off the bottom:
2.0, 7.5 and 13.0 feet
Current meter time in:
8/23/77 at 1512
Current meter time out:
8/29/77 at 1545
Samples taken from top, mid, bottom, included all parameters

Station 73.24C - total depth 12.5 feet

Current meter depth off the bottom:
5 feet
Current meter time in:
8/23/77 at 1440
Current meter time out:
8/27/77 at 1600
Samples taken at mid depth, included all parameters, except kepone

Station 87.67 sampled from 0900 8/24/77 to 1200 8/26/77

Station 87.67 A - total depth 33 feet

Current meter depth off the bottom:
4, 12.5 and 27 feet
Current meter time in:
8/22/77 at 1620
Current meter time out:
8/29/77 at 1423
Samples taken at mid depth, included all parameters except kepone

Station 87.67 B - total depth 23.5 feet

Current meter depth off the bottom:
4, 9.5, and 16 feet
Current meter time in:
8/22/77 at 1705
Current meter time out:
8/29/77 at 1410
Samples taken at top, mid, bottom, included all parameters

Station 87.67 C - total depth 13.5 feet

Current meter depth off the bottom:
5 feet
Current meter time in:
8/22/77 at 1730
Current meter time out:
8/29/77 at 1404
Samples taken at mid depth, included all parameters, except kepone

Station 111 - sampled from 8/24/77 at 0900 to 8/26/77 at 1200

Station 111 A - total depth 18 feet

Current meter depth off the bottom:
4 and 11 feet
Current meter time in:
8/22/77 at 1350
Current meter time out:
8/29/77 at 1215
Samples taken at mid depth, included all parameters
except kepone

Station 111 B - total depth 20 feet

Current meter depth off the bottom:
2, 7.5 and 13 feet
Current meter time in:
8/22/77 at 1140
Current meter time out:
8/29/77 at 1210
Samples taken at top, mid, and bottom depth, included
all parameters

Station 111 C - total depth 13 feet

Current meter depth off the bottom:
5 feet
Current meter time in:
8/22/77 at 1215
Current meter time out:
8/29/77 at 1225
Samples taken at mid depth, included all parameters,
except kepone

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Tide gauges were installed in the following three locations. They were installed one week before the field intensive survey and pulled out one week after the intensive survey. Currently, all tide data are being sent to Fisher and Porter for reduction.

Tide gauge stations:

1) Wooden Pier at Ft. Eustis
2) Pier Chickahominy Holiday Inn Campground (off Rt. 5, near mouth of Chickahominy)
3) Westover, Va. Pier (near Hopewell)

II. Data Reduction

All hydrographical and sediment intensive data are currently being keypunched. Parameters include dissolved oxygen, temperature, conductivity, salinity, suspended solids and kepone concentration. It is anticipated to finish keypunching and editing by the end of November, 1977.

Current meter films have been developed and are being prepared to be read. It is also planned to have the data reduction work done by the end of November, 1977.

PRELIMINARY ANALYSIS OF KEPONE DISTRIBUTION IN THE JAMES RIVER

Donald J. O'Connor
Kevin J. Farley

Environmental Engineering and Science Program
Manhattan College
Bronx, New York 10471

Annual Report to Environmental Protection Agency
Environmental Research Laboratory, Gulf Breeze,
Florida, 1977

Introduction

The general purpose of this research project is to assess the effect of synthetic materials, such as pesticides, on the water quality and ecology of estuarine systems. The present phase of the project is being specifically directed to the analysis of the Kepone distribution in the James River estuary in the vicinity of and downstream from, Hopewell, Virginia. The ultimate goal is to provide a quantitative framework for evaluation of the time required to reduce the Kepone concentrations to acceptable levels.

Significant concentrations of Kepone are present in various phases of the estuarine system of the James River -- in solution, in suspension, in the sediment and in the food chain, particularly in various species of fish. The interrelationships, or more specifically, the transport, uptake and release of Kepone, as shown in Figure II, are thus affected by both physio-chemical mechanisms, as well as bio-ecological phenomena. The former of these includes the hydrodynamic transport through the estuarine system, adsorption to and desorption from the suspended and bed solids, and the settling and resuspension of these solids. The latter incorporates the assimilation and excretion routes through the various components of the food chain. Although less significant for Kepone, transfer to the atmosphere, photochemical oxidation and biological degradation are potentially significant transport and kinetic processes.

Inst 2

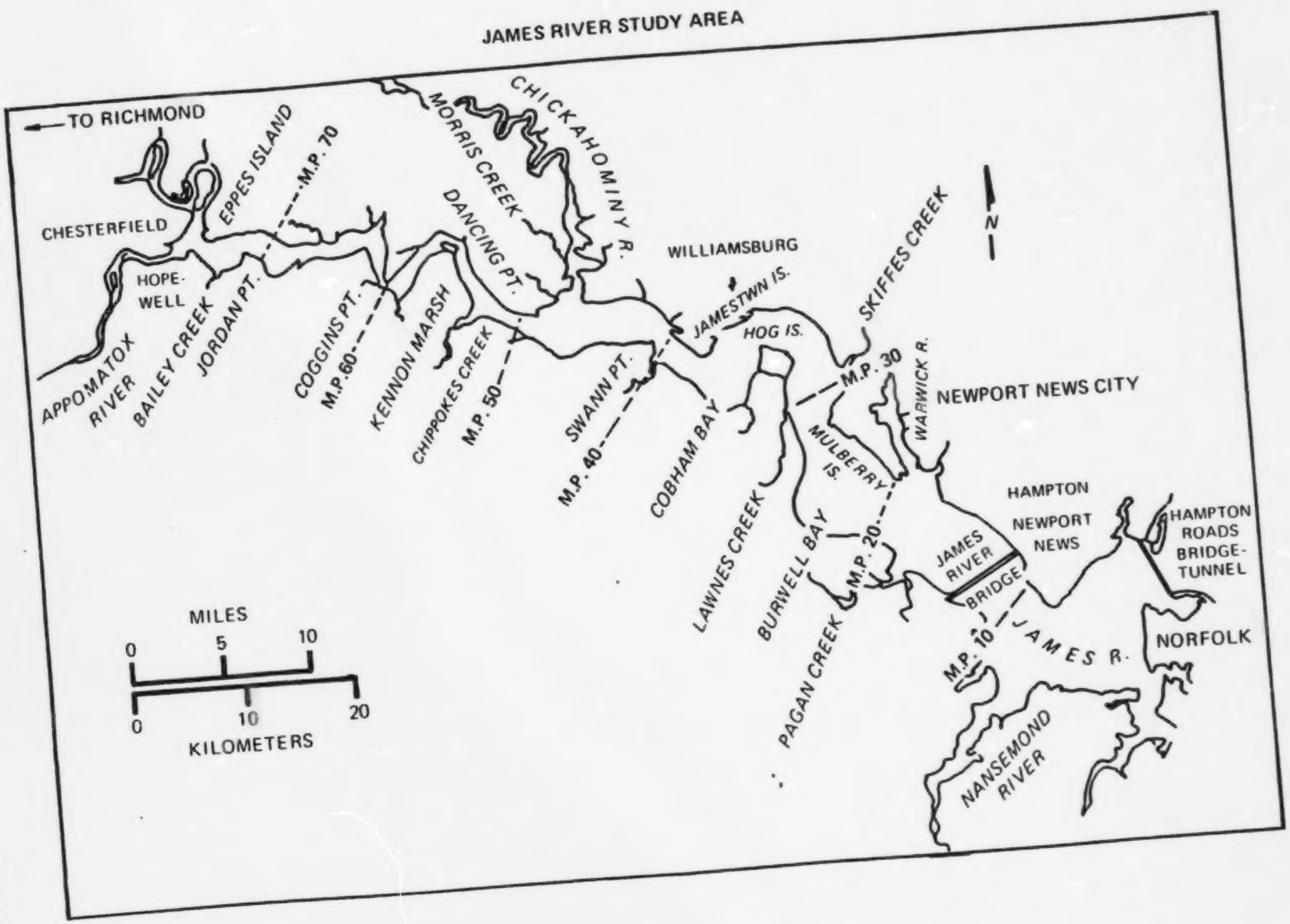

JAMES RIVER STUDY AREA
TO RICHMOND
CHESTERFIELD
EPPES ISLAND
M.P. 70
HOPE-WELL
APPOMATOX RIVER
BAILEY CREEK
JORDAN PT.
COGGINS PT.
M.P. 60
KENNON MARSH
CHIPPOKES CREEK
M.P. 50
DANCING PT.
MORRIS CREEK
CHICKAHOMINY R.
WILLIAMSBURG
JAMESTWN IS.
HOG IS.
SWANN PT.
M.P. 40
COBHAM BAY
LAWNES CREEK
SKIFFES CREEK
M.P. 30
WARWICK R.
NEWPORT NEWS CITY
MULBERRY IS.
BURWELL BAY
M.P. 20
PAGAN CREEK
HAMPTON
NEWPORT NEWS
JAMES RIVER BRIDGE
M.P. 10
JAMES R.
HAMPTON ROADS BRIDGE-TUNNEL
NORFOLK
NANSEMOND RIVER
N
MILES
0
5
10
0
10
20
KILOMETERS

FIGURE I
JAMES RIVER STUDY AREA

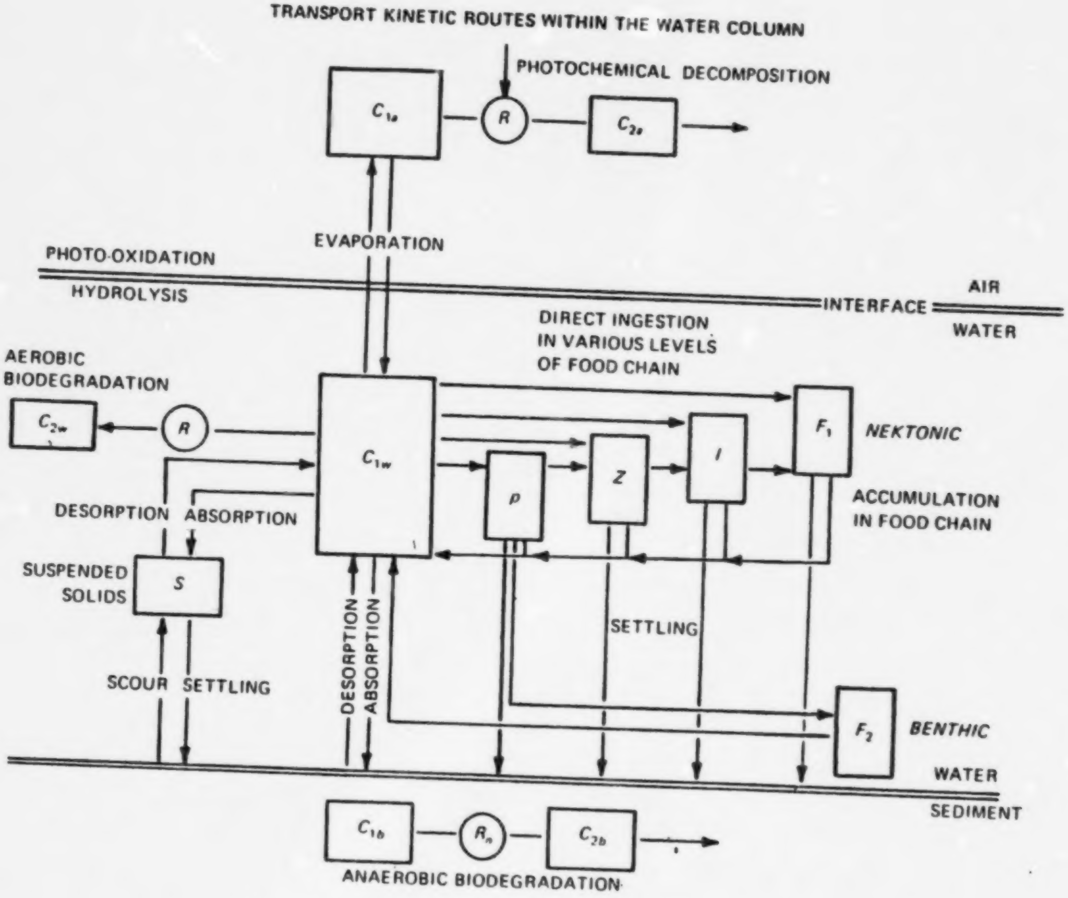

TRANSPORT KINETIC ROUTES WITHIN THE WATER COLUMN
PHOTOCHEMICAL DECOMPOSITION
C_{1a}
R
C_{2a}
EVAPORATION
PHOTO-OXIDATION
HYDROLYSIS
INTERFACE
AIR
WATER
DIRECT INGESTION IN VARIOUS LEVELS OF FOOD CHAIN
AEROBIC BIODEGRADATION
C_{2w}
R
C_{1w}
p
Z
I
F_1
NEKTONIC
ACCUMULATION IN FOOD CHAIN
DESORPTION
ABSORPTION
SUSPENDED SOLIDS
S
SCOUR
SETTLING
DESORPTION
ABSORPTION
SETTLING
F_2
BENTHIC
WATER
SEDIMENT
C_{1b}
R_n
C_{2b}
ANAEROBIC BIODEGRADATION

FIGURE $\overline{\underline{II}}$

TRANSPORT - KINETIC ROUTES WITHIN THE WATER COLUMN

The Distribution of Kepone on Solids

Natural clays of various types, and organic material, possess an adsorptive capacity. The rates of adsorptive reactions are being investigated experimentally under controlled laboratory conditions in order to provide realistic kinetic coefficients for the Kepone analysis. The desorptive characteristics of both the inorganic and organic fractions of the suspended solids are also being reviewed. This phenomena of adsorption-desorption is one of the important transfer routes in the ultimate transfer of Kepone from the system. Based on the Langmuir Isotherms, equations have been developed to predict the spatial and temporal distributions of Kepone in an advective-dispersive estuarine system. However, due to the preliminary nature of this work, the less complex, advective, steady state model was used for analysis. Equations governing the water column and estuarine bed for such a system are as follows:

1. Water

Solids $U_1 \frac{\partial m_1}{\partial x} = -K_s m_1 + \alpha K_u m_2$

Dissolved $U_1 \frac{\partial C_1}{\partial x} = -K_o(r_c - r_1)m_1 C_1 + K_d r_1 m_1 - K_b(C_1 - C_2) - K_a C_1$

Particulate $U_1 \frac{\partial P_1}{\partial x} = +K_o(r_c - r_1)m_1 C_1 - K_d r_1 m_1 - K_s r_1 m_1 + \alpha K_u r_2(m_2 - m_1)$

2. Bed

Solids $U_2 \frac{\partial m_2}{\partial x} = + \frac{K_s}{\alpha} m_1 - K_u m_2$

Dissolved $$U_2 \frac{\partial C_2}{\partial x} = -K_o(r_c-r_2)m_2C_2+K_d r_2 m_2+K_b(C_1-C_2)$$

Particulate $$U_2 \frac{\partial P_2}{\partial x} = +K_o(r_c-r_2)m_2C_2-K_d r_2 m_2+\frac{Ks}{\alpha}r_1 m_1-K_u r_2(m_2-m_1)$$

where:

the subscripts 1 and 2 denote the water column and estuarine bed concentrations, respectfully,

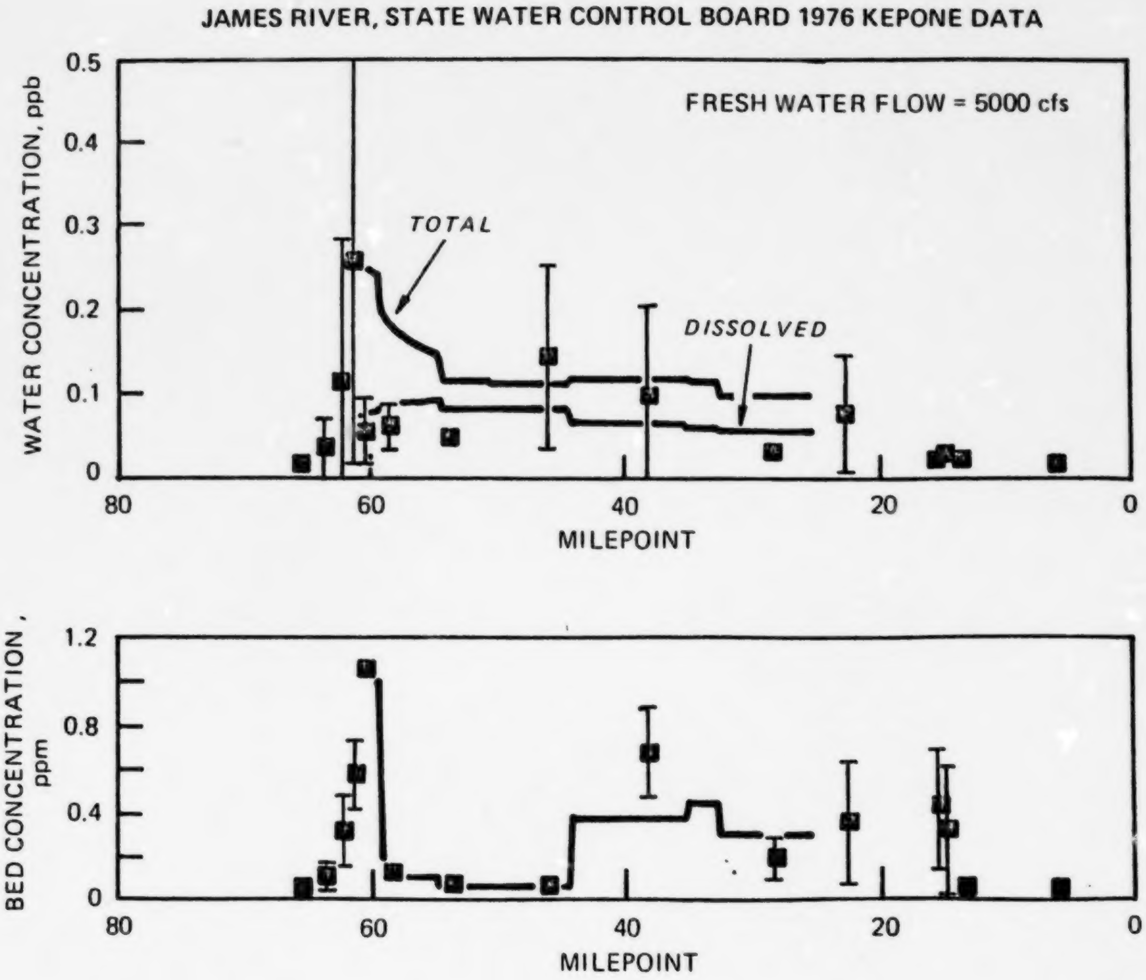
and where:

U - horizontal velocity [m/sec]
C - dissolved Kepone concentration [μg/ℓ]
x - longitudinal distance [meters]
K_o - adsorption coefficient [1/(μg/ℓ-day)]
r_c - solids adsorptive capacity [μg/g]
r - Kepone concentration on the solids [μg/g]
m - solids concentration [g/ℓ]
K_d - desorption coefficient [1/day]
K_b - bed diffusion coefficient [1/day]
K_a - aeration coefficient [1/day]
P - solids Kepone concentration [μg/g]
K_s - solids settling coefficient [1/day]
α - the ratio of bed volume to water column volume [dimensionless]
K_u - solids scour coefficient [1/day]

As a first step, this preliminary analysis was simplified by various assumptions - subject to verification by the ongoing field and laboratory studies. The first of these assumptions - solids being in equilibrium i.e. $\frac{\partial m_1}{\partial x}$ and $\frac{\partial m_2}{\partial x} = 0$, appears to be a safe assumption for the non-saline portion of the estuary. In addition, the bed solids concentration, m_2, was said to be much greater than the suspended solids concentration, m_1; the aeration term, K_a, was taken to be negligible; and the solids adsorptive capacity, r_c, was assumed to be much greater than either of the Kepone concentrations on the solids, r_1 and r_2. The kinetic coefficients - K_o, K_d, K_s, and K_u, were assigned from the limited data available. Finally, for this "first-cut" model, the Kepone concentrations on the bed solids, r_2, were assigned from data; these concentrations were in turn utilized in predicting the Kepone water column concentrations.

Based on these assignments of coefficients, the longitudinal distribution of total and dissolved Kepone in the water column is presented in Figure III along with the State Water Control Board 1976 Kepone data. The line of total Kepone concentration fits the data quite well and although the dissolved fraction of Kepone is high, this concentration is merely a function of Kepone kinetic coefficients, K_o and K_d - values which were obtained from a minimal amount of sketchy data. Further analysis is presently being performed which will predict both the water column and the bed concentrations of Kepone.

The above analysis will be further complicated as the



JAMES RIVER, STATE WATER CONTROL BOARD 1976 KEPONE DATA

FIGURE III

KEPONE CALCULATION FOR THE JAMES RIVER ESTUARY (1976) saline portion of the estuary is approached. As the lighter clay particles which are maintained in suspension in the non-saline area encounter the saline region of the estuary, flocculation and agglomeration may occur, increasing the size and possibly the density of the particles. These factors result in further deposition, which is enhanced by virtue of their occurrence in the null zone of the estuary. There are, therefore, a variety of significant factors which may account for the accumulation of solids and Kepone in the estuarine bed at the fresh water-saline interface. These factors, along with the inability to assume solids equilibrium in the saline region, have lead to a detailed investigation of solid material in the estuary.

Hydrodynamic Transport

Since the concentration of suspended solids is an important factor as an accumulation site for Kepone, the temporal and spatial distribution of the solids within the estuarine system is a necessary element in the analysis. The distribution is determined by the hydraulic transport through the estuarine system. A two-dimensional (longitudinal-vertical) analysis has been developed, based on the fundamental principles of momentum, continuity and state.

In this analysis, under steady state, tidally averaged conditions, the longitudinal momentum equation for a laterally homogeneous estuary is:

$$0 = \frac{1}{\rho}\frac{\partial p}{\partial x} + N\frac{\partial^2 u}{\partial z^2} \tag{1}$$

where ρ = density; p = pressure; N = vertical eddy viscosity; and u = horizontal velocity. The coordinates for Eq. 1 are shown in Fig. IV in which the longitudinal x-axis is positive toward the ocean and the vertical z-axis is positive toward the bed of the estuary channel. Boundary conditions compatible with Eq. 1 are,

$$\frac{\partial u}{\partial z} = 0 \qquad \text{at } z = -n \tag{2}$$

$$-N\frac{\partial u}{\partial z} = C_d/u_b/u_b \qquad \text{at } z = h \tag{3}$$

in which -n = surface elevation and h = average depth; C_d = dimensionless friction coefficient; and u_b = velocity at the bed. The vertical component of the momentum equation is simply the hydrostatic pressure equation:

$$\frac{1}{\rho}\frac{\partial p}{\partial z} = g \tag{4}$$

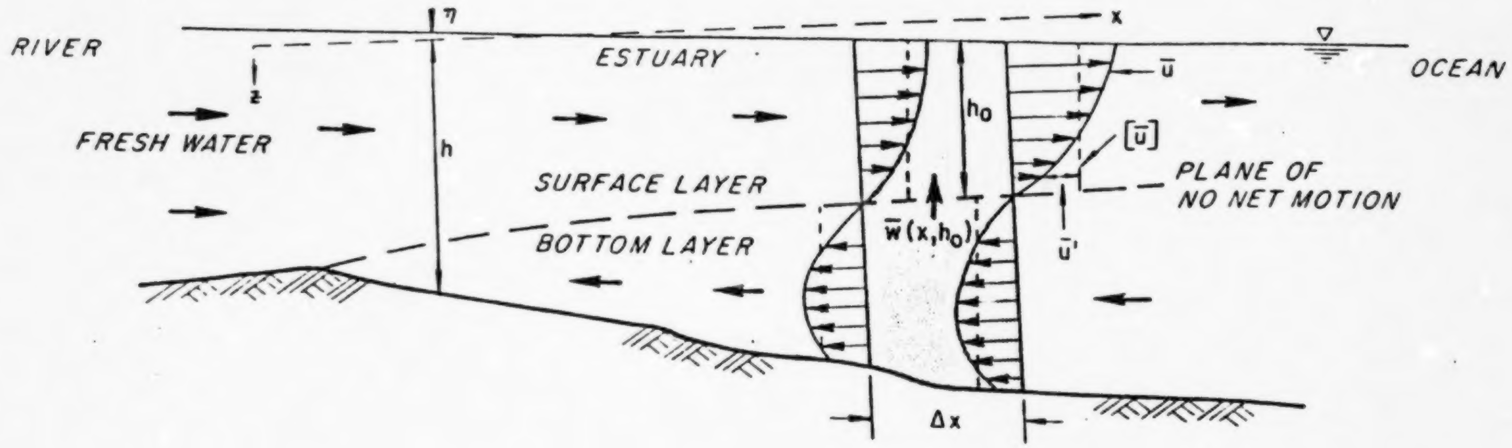

RIVER
FRESH WATER
ESTUARY
SURFACE LAYER
BOTTOM LAYER
OCEAN
PLANE OF NO NET MOTION
η
x
z
h
h_0
$\bar{w}(x,h_0)$
$\bar{u}$
$[\bar{u}]$
$\bar{u}'$
Δx

FIGURE $\overline{\text{IV}}$

SCHEMATIC DIAGRAM OF TWO-DIMENSIONAL ESTUARINE CIRCULATION

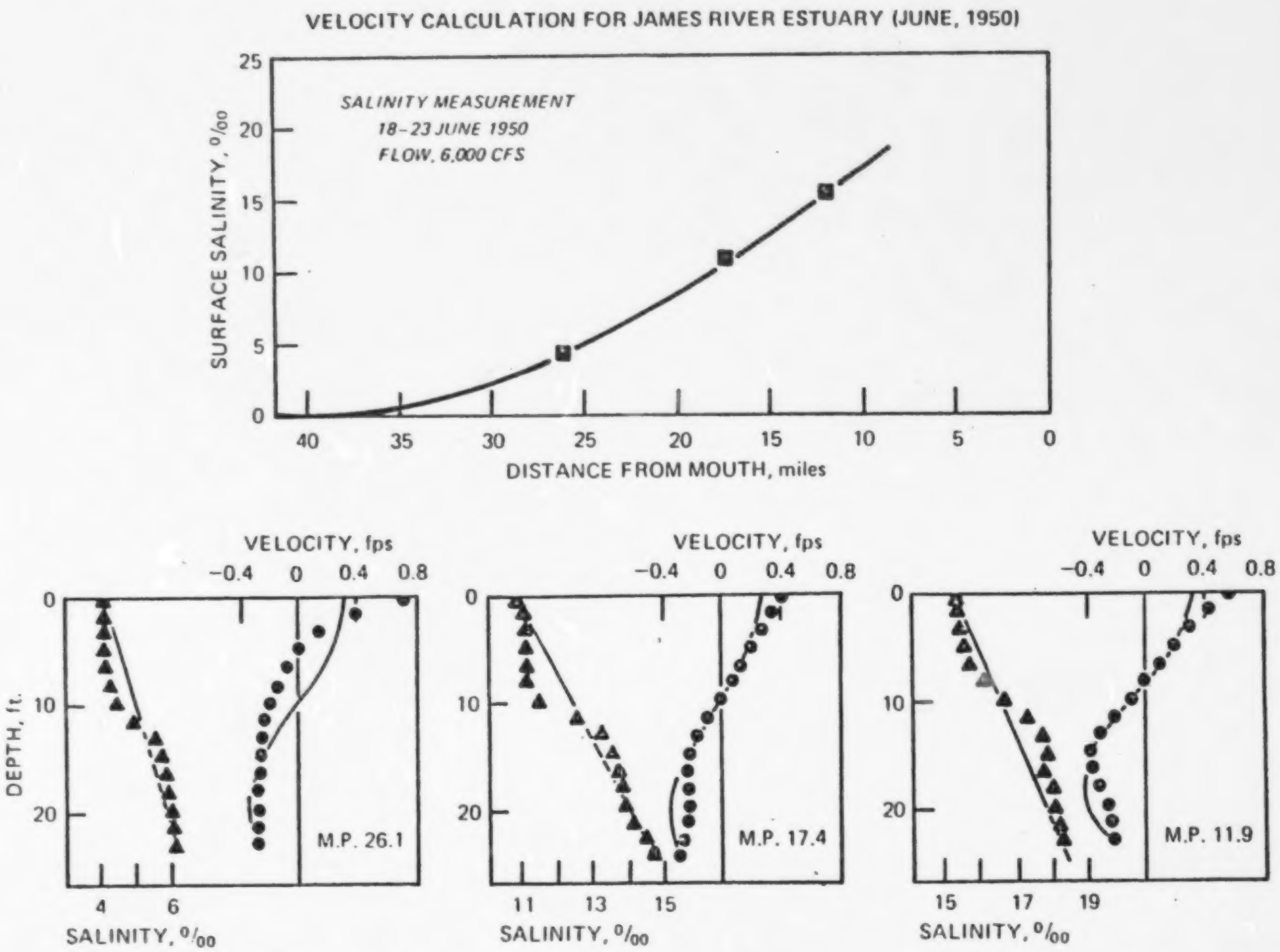
In order to solve Eq. 1, the hydrostatic pressure, Eq. 4, is expressed in terms of the horizontal and vertical distribution of salinity. The equation of state which specifies the density as a function of salinity is given by:

$$\rho = \rho_f(1 + \alpha\bar{C}) \tag{5}$$

in which ρ_f = the density at zero salt content and $\alpha = 0.000757$ (parts per thousand)$^{-1}$. The components of the pressure force are then evaluated in terms of the observed vertical and longitudinal salinity gradients and freshwater flow, which are assumed known from measurement.

The solution of the above equations indicates that local rather than boundary conditions control the magnitude and gradient of horizontal velocity at a particular location. Because of local control, the velocity at one location is relatively independent of those at other locations. This condition occurs as a result of decoupling the equations of motion and salt transport.

Results of this analysis are presented for Pritchard's June 1950 survey and Nichols' March 1965 survey of the James River in Figure V and VII respectfully. In addition, the solution also indicates the depth at which the net horizontal velocity is zero. Defining this depth at a number of stations and interpolating for others delineates the plane of no net motion for the saline intrusion zone of the estuary, Figures VI and VIII. At the tail of the salinity intrusion, this plane meets the bed of



NOTE: SALINITY AND VELOCITY MEASUREMENTS BY THE CHESAPEAKE BAY INSTITUTE

FIGURE $\overline{V}$

VELOCITY CALCULATION FOR JAMES RIVER ESTUARY (JUNE, 1950)

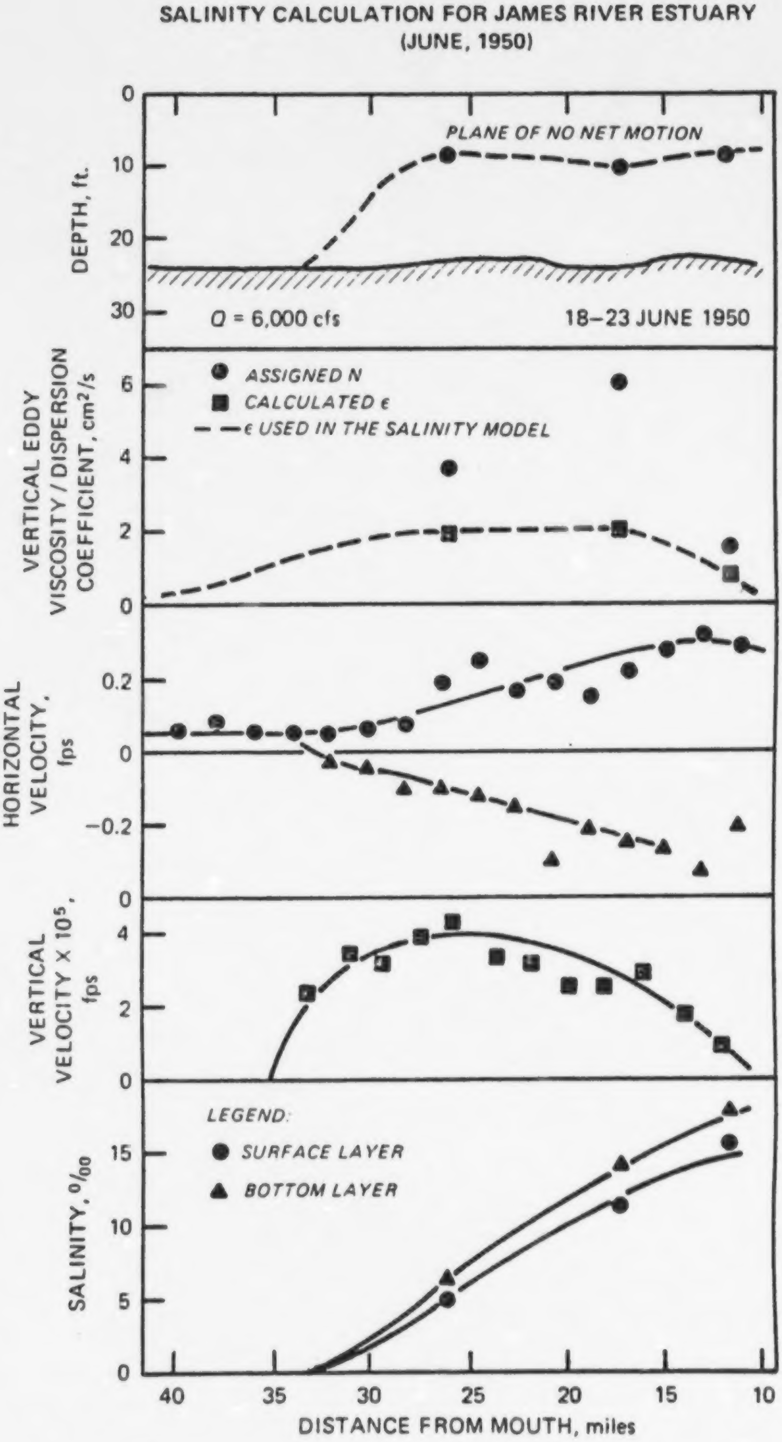
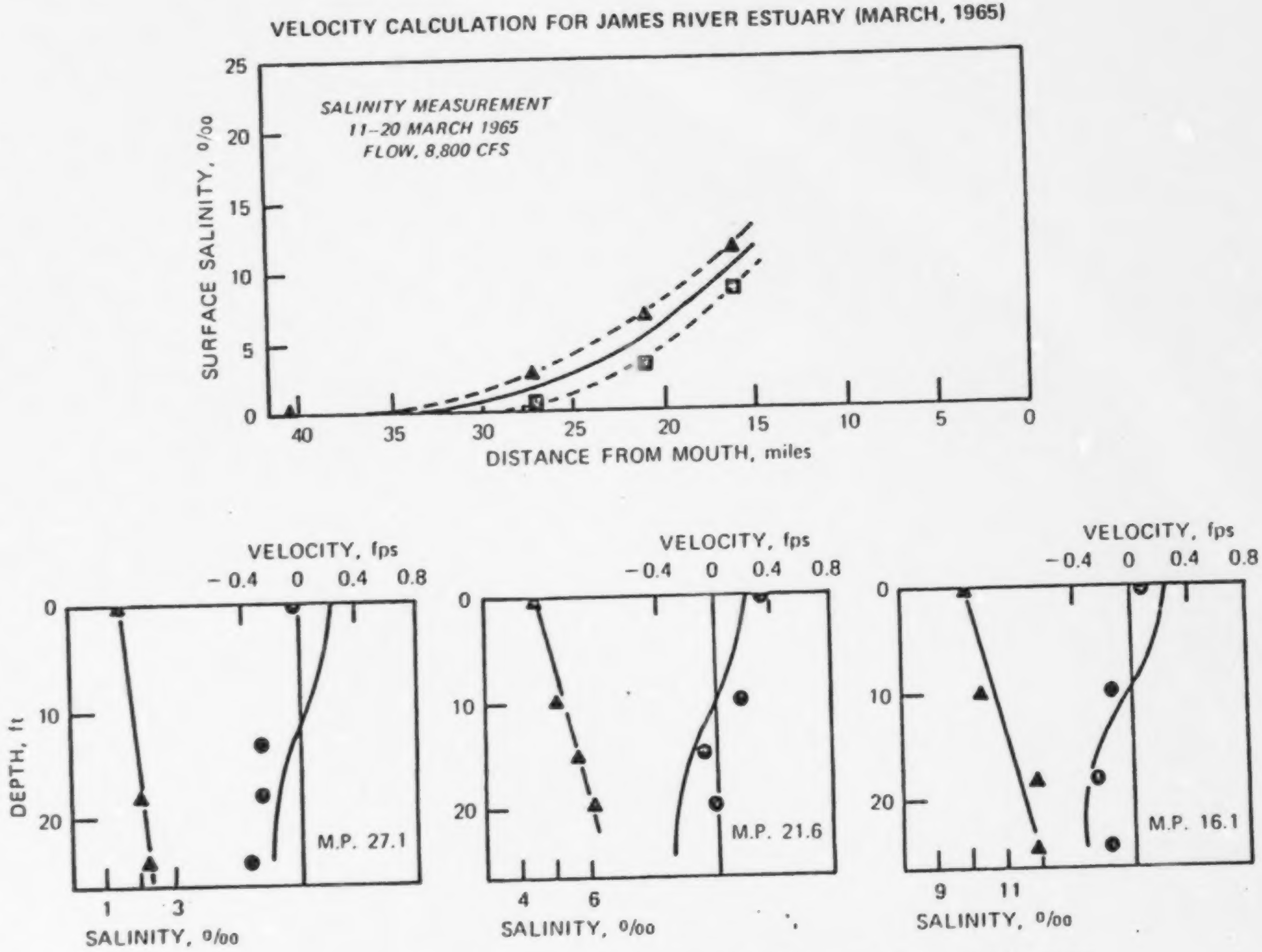


FIGURE VI

SALINITY CALCULATION FOR JAMES RIVER ESTUARY (JUNE, 1950)



NOTE: SALINITY AND VELOCITY MEASUREMENTS BY THE VIRGINIA INSTITUTE OF MARINE SCIENCE

FIGURE $\overline{\text{VII}}$

VELOCITY CALCULATION FOR JAMES RIVER ESTUARY (MARCH, 1965)

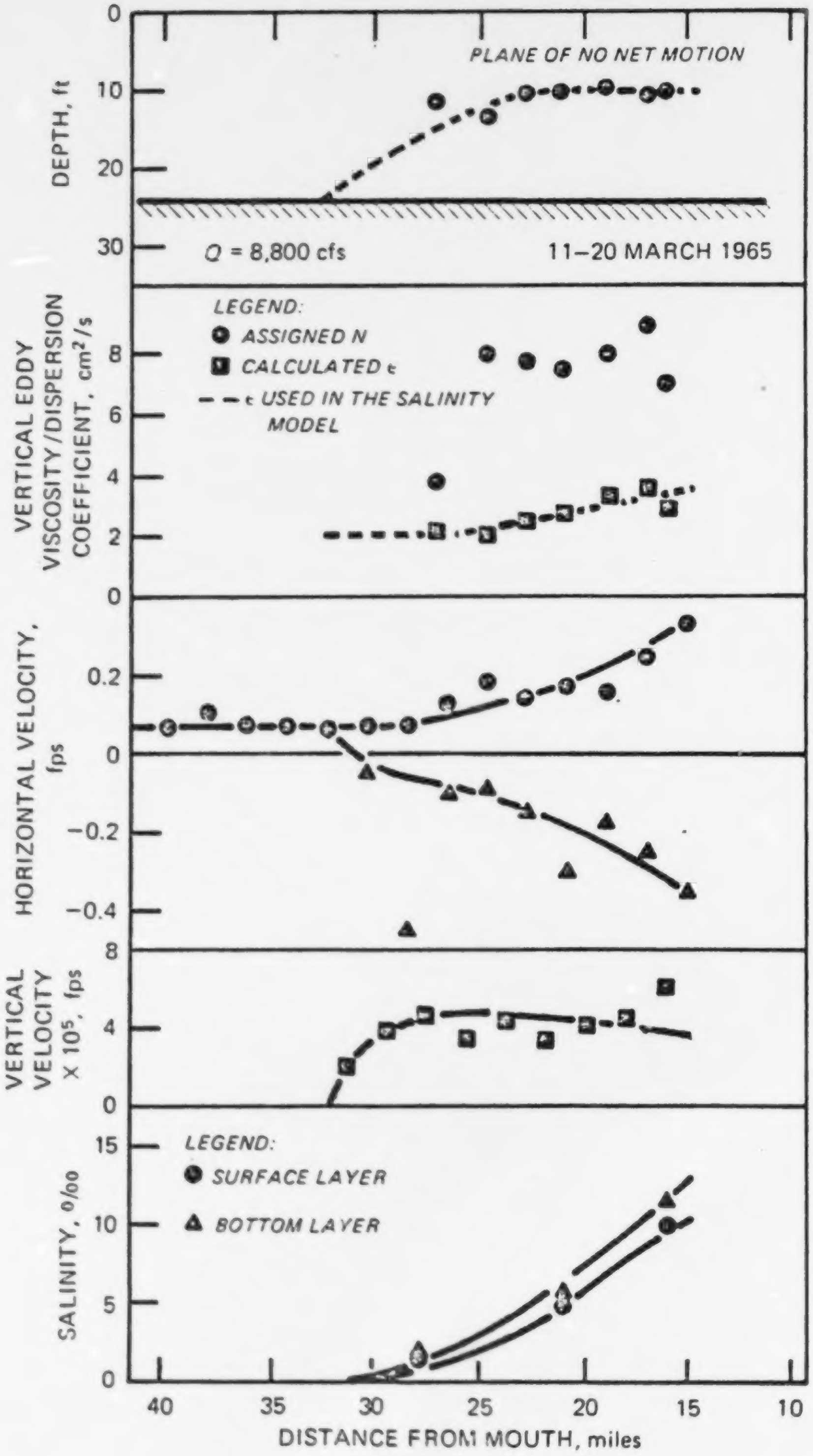


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FIGURE VIII

SALINITY CALCULATION FOR JAMES RIVER ESTUARY (MARCH, 1965) the estuary. Upstream of this area, the horizontal velocity in the whole water column is in the seaward direction.

The estuary is then segmented horizontally and the horizontal flow in the surface layer at each vertical cross section is first calculated. Horizontal flow difference between two adjacent vertical planes gives the vertical flow between the surface and bottom layer, from which the vertical velocity is obtained by dividing by the average width of the segment. This procedure is obviously a solution of the hydraulic continuity.

The vertical flux of salt due to dispersion between the surface and bottom layers is described by the dispersion coefficient, ε, obtained from the vertical eddy viscosity through an empirical relationship,

$$\varepsilon = N(1 + R_1)^{-1} \tag{6}$$

where Ri (Richardson number) is defined as:

$$Ri = \frac{g\frac{\partial \rho}{\partial z}}{\rho(\frac{\partial \bar{u}}{\partial z})^2} \tag{7}$$

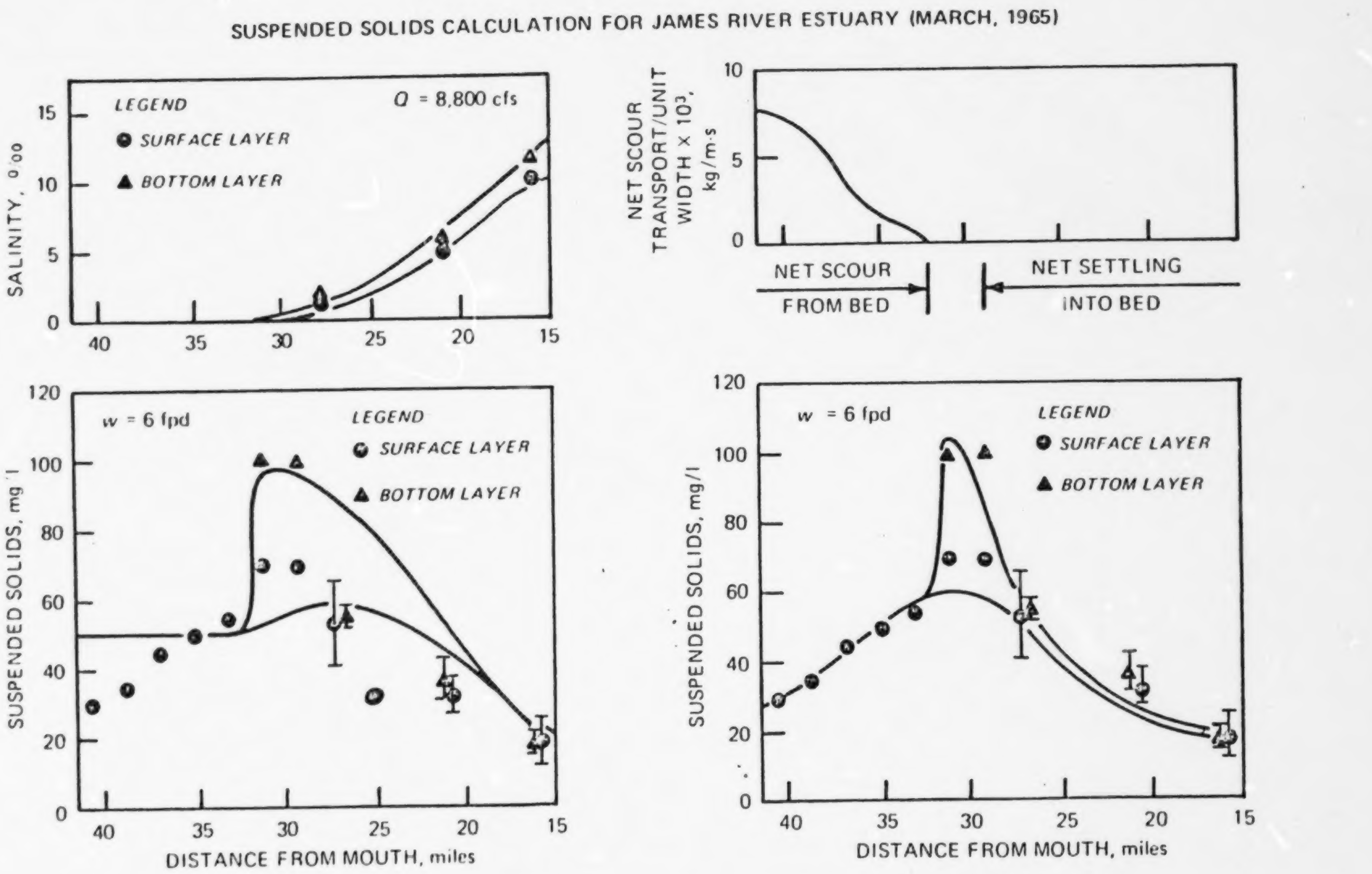
Equation 6 indicates the relationship between the two coefficients, whose general validity has been shown by field data, as presented by Officer.

The tidal diffusion and velocity shear contributions, which can be envisioned collectively as a longitudinal dispersion across a vertical section following the classical one-dimensional estuarine analysis, did not exhibit themselves in the portion of the estuary that our models were concerned.

The distribution of salinity was used to test the validity of the hydrodynamic model - bottom panels of Figures VI and VIII. Based on these validations of the hydrodynamic model, an analysis of suspended solids followed by incorporating the settling and scour rates with the hydrodynamic transport to determine the distribution of solids. Settling rates, for the present, were assumed constant down the length of the estuary and this rate was obtained from the average particle size, using a modification of Stokes' Law. Since little work has yet been performed on scouring rates in estuaries, these rates were assigned merely to show that a good fit can be obtained. Results of this solids modeling, with and without the assigned scouring rates, are presented in Figure IX.

ASSIMILATION AND DEPURATION OF KEPONE IN THE FOOD CHAIN

The transfer of Kepone from its initial discharge at Hopewell to its accumulation in the fishery stock may occur in a number of ways. It may be ingested directly from that which is dissolved or suspended in the water; it may be assimilated by the phytoplankton-zooplankton; and it may be taken in by bottom feeders from the material which has settled in the channel bed. The predominant sites for settling appear to be downstream from Hopewell, in the region of the fresh water-saline interface, and in various dead zones in the fresh and saline regions. Experiments involving assimilation and depuration of Kepone by various species are being conducted. The rates of accumulation and



NOTE: SUSPENDED SOLIDS MEASUREMENTS BY THE VIRGINIA INSTITUTE OF MARINE SCIENCE

FIGURE IX

SUSPENDED SOLIDS CALCULATION FOR JAMES RIVER ESTUARY (MARCH, 1965) excretion, equilibrium conditions and concentrations, lethal and chronic - are being analyzed in order to incorporate these kinetic factors in a food chain analysis.

Preliminary analysis has been made in evaluating the assimilation and depuration kinetics on various species of fish. Data from experimental studies performed at EPA's Gulf Breeze Laboratory are used to evaluate the relevant coefficients. The equation utilized in this analysis - similar to the Langmuir kinetic equation for the adsorption to and desorption from suspended solids, is as follows:

$$\frac{\partial(rm)}{\partial t} = K_o(r_c - r)m(t)C - K_d rm(t)$$

where

r - Kepone concentration in the biomass [μg/g]

m - biomass concentration [g/ℓ]

t - time [days]

K_o - assimilation coefficient [1/day]

r_c - biomass assimilation capacity [μg/g]

C - dissolved Kepone concentration [μg/ℓ]

K_d - depuration coefficient [1/day]

The only assumption made in this analysis was that the biomass assimilation capacity, r_c, was taken to be much greater than the Kepone concentration in the biomass, r. Results of this analysis for oysters (Crassostrea, virginica) are presented in Figure X.

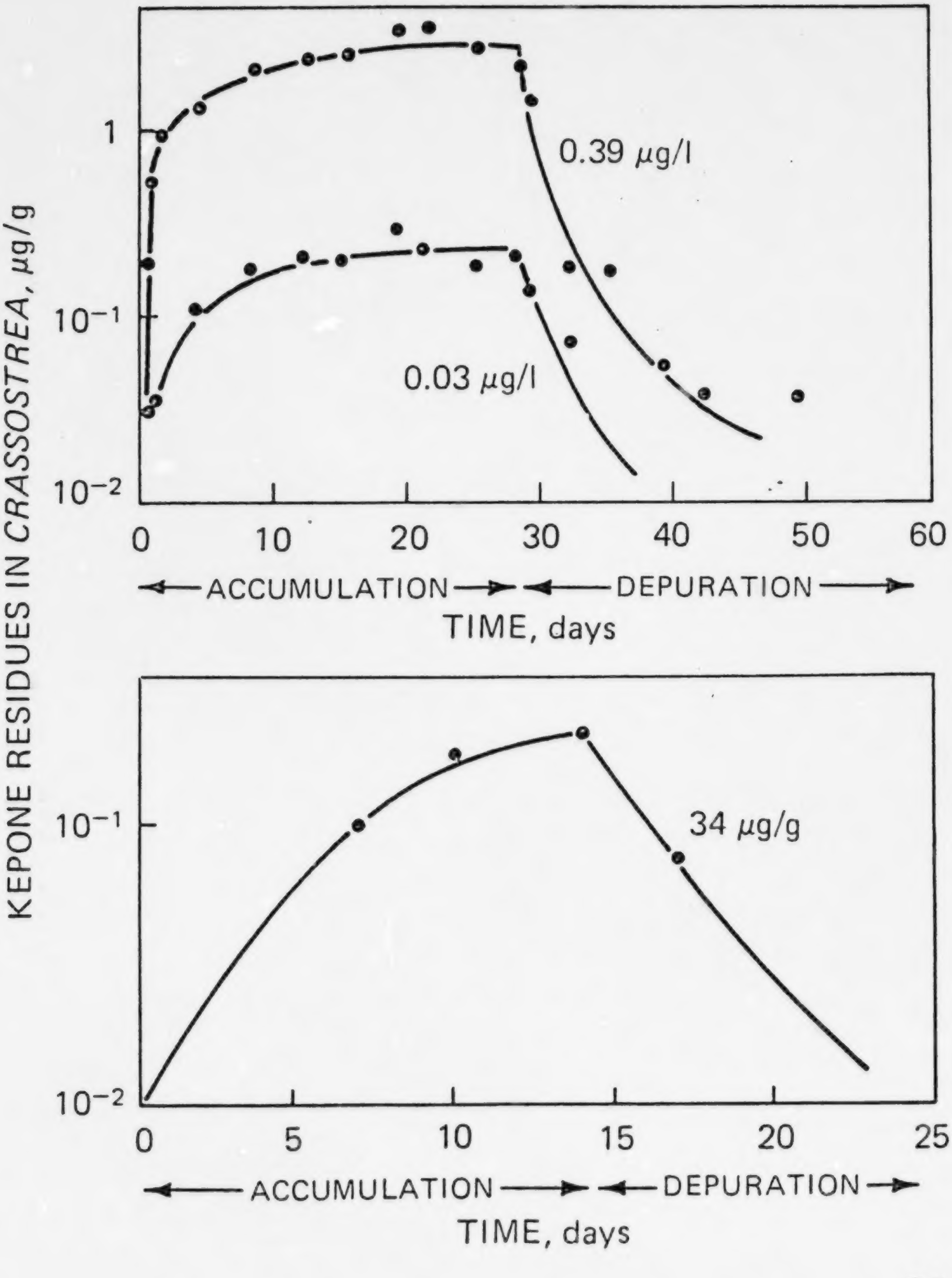


FIGURE X

CALCULATION FOR THE ASSIMILATION AND DEPURATION OF KEPONE IN OYSTERS

From these results, it can be shown that the bio-ecological phenomena of assimilation and depuration can be modeled utilizing Langmuir kinetics if data for the evaluation of the relevant coefficients is available.

CONCLUSION

The equations presented in this report appear to be sufficiently realistic as a first approximation in representing the various phenomena under consideration. At the present time, the analysis is being extended to treat the ecological system as a continuum using trophic length as a metric. Given the inputs from the sources in the vicinity of Bailey's Bay, the transport in the non-saline and saline regions of the James estuary and the distribution of suspended solids and Kepone, the food chain model is being enlarged to include the uptake and excretion of Kepone in the various trophic levels and the predation and feeding associated with these levels. At this time, the saline and non-saline regions of the estuary are being combined into one continuous solution. Steady state conditions, which represent average conditions during various seasons of the year, are being assumed for these preliminary steps of the analysis.

ACKNOWLEDGEMENTS

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Schnoor is acknowledged. Various phases of the computations were performed by Cherng-Ju Kim and George A. Leahy, research assistants in the Environmental Engineering and Science Program at Manhattan College.

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Technical Report No. 35

SURVIVAL, DURATION OF LARVAL STAGES, AND SIZE OF POSTLARVAE OF GRASS SHRIMP, *PALAEMONETES PUGIO*, REARED FROM KEPONE® CONTAMINATED AND UNCONTAMINATED POPULATIONS IN CHESAPEAKE BAY

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SURVIVAL, DURATION OF LARVAL STAGES, AND SIZE OF POSTLARVAE OF GRASS SHRIMP, *PALAEMONETES PUGIO*, REARED FROM KEPONE® CONTAMINATED AND UNCONTAMINATED POPULATIONS IN CHESAPEAKE BAY

I. INTRODUCTION

Discharge of Kepone® (chlordecone) into the James River at Hopewell, Virginia has created far reaching environmental, economic, and potential health problems for the people of Virginia and neighboring areas. Kepone, like other chlorinated insecticides, is highly cumulative and persists in estuarine organisms. Oysters, grass shrimp, and fishes have concentrated Kepone from 425 to 20,000 times the concentration in surrounding water (Hansen, et. al., 1976).

Palaemonetes pugio is one of the key components in Atlantic coast estuaries. A major fraction of the total energy flow through the food web of these estuarine communities passes through its populations (Welsh, 1975). In 1975, broods of larvae of *P. pugio* obtained from stocks in the Lafayette River, Norfolk, Virginia, were reared in the laboratory of the Institute of Oceanography, Old Dominion University, in an attempt to define the environmental requirements for the larvae and to establish conditions necessary for consistent success. Erratic results under apparently constant laboratory conditions were obtained. Samples of *P. pugio* from the same locality sent to the Environmental Research Laboratory, Gulf Breeze, Florida for experimentation, were found to contain significant amounts of Kepone, suggesting the possibility that Kepone may affect larval survival.

The present study was undertaken to determine whether, under standard laboratory conditions, broods of larvae of this species obtained from Kepone contaminated and uncontaminated sites in the Chesapeake Bay might vary in larval survival, time required for larval development, and size of postlarvae. Inasmuch as variability among females had not been established, an attempt was made to evaluate the contribution of this source of variation as well.

II. METHODS

Ovigerous grass shrimp were collected with a pushnet from six Virginia localities in the Chesapeake Bay region (fig. 1): (1) the Lafayette River in urban Norfolk, (2) bridge pilings at Gloucester Point, at the mouth of the York River, (3) Lynnhaven Inlet, Virginia Beach, at the Chesapeake Bay mouth, (4) small inlet at Fleeton, Virginia, near the mouth of the Potomac River, (5) the James River Bridge, Portsmouth, Virginia, and (6) the western end of the Route 301 bridge across the Potomac River. These localities were selected in an attempt to include Kepone contaminated and uncontaminated sites, but the results of analyses establishing Kepone levels in the populations from these sites were not available until well after the rearing experiments were completed.

Females with embryos in advanced state of development were sorted from the general catch and placed into individual hatching units. Each unit was maintained in a separate one-gallon (3.8-liter) aerated aquarium containing filtered natural seawater (25 ^{o}/oo S) at ambient temperature (22° to 26° C). Water was changed daily, and hatching usually occurred within 48 hours after collection. Hatching units were constructed of 10-cm lengths of 7-cm diameter clear plastic tubing (fig. 2). A layer of plastic mesh (onion bag netting) was stretched across the bottom of each tube and attached with aquarium sealant; a second layer (several centimeters above the bottom) supported the female. The double mesh prevented the female from capturing and eating her newly hatched larvae which escaped through the bottom of the unit.

Replicate groups of 33 larvae were obtained from each of the three isolated females from each site; each group was placed into a 20-cm-diameter glass culture dish containing one liter of artificial seawater (Instant Ocean, Aquarium Systems, Inc. Eastlake,

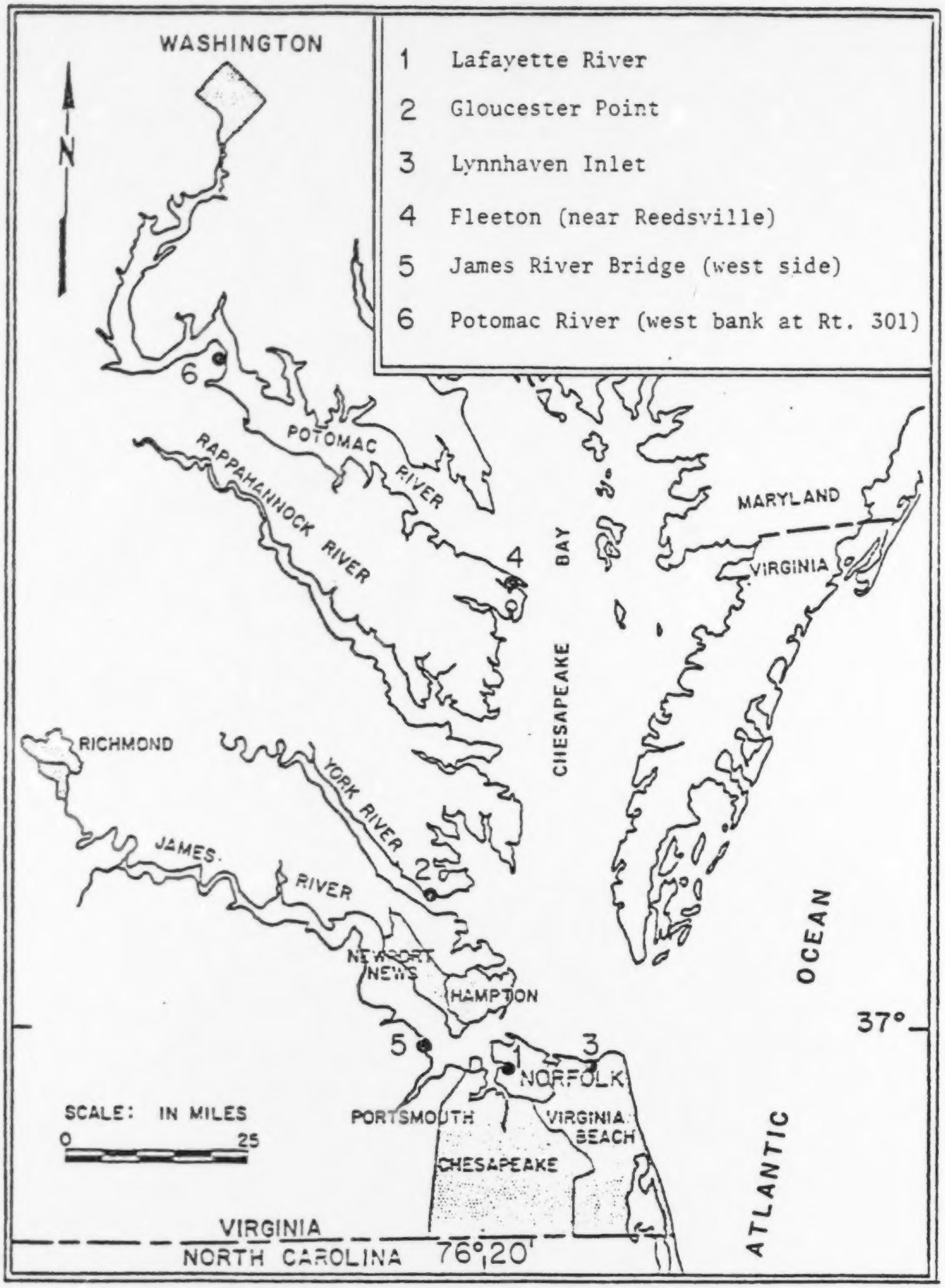
WASHINGTON
N
1 Lafayette River
2 Gloucester Point
3 Lynnhaven Inlet
4 Fleeton (near Reedsville)
5 James River Bridge (west side)
6 Potomac River (west bank at Rt. 301)
6
POTOMAC RIVER
RAPPAHANNOCK RIVER
4
CHESAPEAKE BAY
MARYLAND
VIRGINIA
RICHMOND
YORK RIVER
JAMES RIVER
2
NEWPORT NEWS
HAMPTON
OCEAN
37°
5
1
3
NORFOLK
SCALE: IN MILES
0
25
PORTSMOUTH
VIRGINIA BEACH
CHESAPEAKE
ATLANTIC
VIRGINIA
NORTH CAROLINA
76°20'

Figure 1. Location of collection sites.

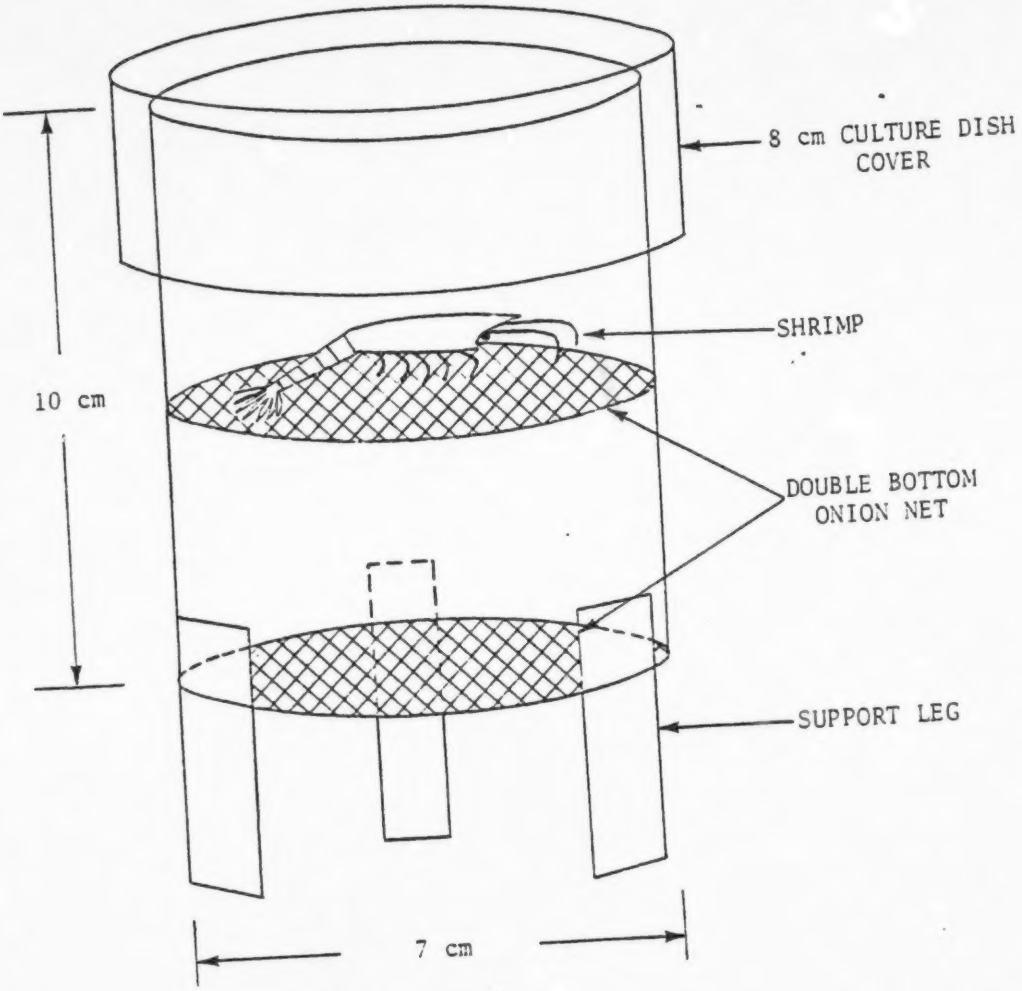
8 cm CULTURE DISH COVER
SHRIMP
10 cm
DOUBLE BOTTOM ONION NET
SUPPORT LEG
7 cm

Figure 2. Hatching unit.

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Ohio) at 25 ppt prepared with tap water. Larvae from each female were reared separately in order to estimate variation due to parentage. Thus, six dishes, each with 33 larvae, represented each site.

Larvae were reared in an incubator at 25° C in the dark. Food consisted of freshly hatched San Francisco Bay Brand *Artemia* nauplii at an initial daily concentration of 10 nauplii/ml of medium. Larvae were observed, counted, and changed to freshly prepared bowls each day.

Newly metamorphosed post larvae were removed daily, measured from tip of nostrum to tip of telson (using a dissecting microscope and a millimeter rule, estimated error = ± 0.2 mm), and frozen for Kepone analysis.

From each collection site the following samples were frozen and transported to the Virginia Institute of Marine Science, Gloucester Point, for Kepone analysis:

a. Ovigerous females
b. Eggs removed from the pleopods of additional females
c. Non-ovigerous females which had recently hatched larvae
d. Larvae newly hatched in the laboratory
e. Laboratory reared post larvae.

Analytical Methods
(Adapted from report by M. Keith Ward)

Frozen samples were delivered to the Virginia Institute of Marine Science during the period through August 1976 for Kepone analysis. One third of the samples were analyzed during the period July through September 1976 and the remainder in February 1977.

Each total sample was extracted with three successive 20-ml portions of toluene in ethylacetate (1:4) using a Polytron. The Polytron generator head was rinsed with a few milliliters of extracting solvent to minimize Kepone loss. The sample tube was centrifuged after each extraction and the supernatant solution was passed through anhydrous granular sodium sulfate into a 50-ml screwcap culture tube. The extract was concentrated to less than 1 ml using a gentle stream of nitrogen and a warm water bath.

A Florisil column consisting of 1.6 gram Florisil covered by 1.6 gram anhydrous granular sodium sulfate in a glass column (dimensions 100 mm × 30 mm, Corning #412160) was used in an attempt to remove components that might interfere with Kepone. The Florisil column was wet with Solvent I (2 percent methanol, 4 percent benzene, in hexane). The sample was transferred to the column and the sample tube rinsed five times with 0.5 ml Solvent I. Seven milliliters of Solvent I was collected as Fraction I and discarded. This was followed by 30 ml Solvent II (Fraction II) consisting of 1 percent methanol, 2 percent acetonitrile, 4 percent benzene in hexane. Fraction II (which contains Kepone) was analyzed for Kepone by electron capture gas chromatography.

The *P. pugio* samples were quantitated for Kepone on either of two gas chromatographs: a Varian Model 2700 equipped with two ^3H(tritium) electron capture detectors operated at 220° C, and a column temperature of 210° C; or a Tracor Model 222 equipped with two linearized high-temperature ^{63}Ni detectors operated at 350° C and a column temperature of 210° C. The samples were analyzed on at least two of the following columns: 3 percent OV-1; 1.5 percent OV-17 + 1.95 percent OV-210, or 4 percent SE-30 + 6 percent OV-210, on 80 to 100 mesh Gas Chrom Q or Variaport 30. The column dimensions were 6 mm × 2 mm × 183 mm. Values for Kepone concentrations presented in table 1 represent the mean of two readings.

Statistical Analyses

Three parameters were analyzed statistically in order to evaluate intrasite variation due to parentage and intersite variation of the laboratory-reared larvae. These were (1) survival of laboratory-reared larvae, (2) time to metamorphosis (larval duration), and (3) length of postlarvae (rostrum-telson) at metamorphosis.

Table 1. Concentration of Kepone (ppm) in samples of *P. pugio* arranged by collection site (courtesy of Virginia Institute of Marine Science).

	Site					
	1 Lafayette River	2 Gloucester Point	3 Lynnhaven	4 Fleeton	5 James River	6 Potomac River
Ovigerous females	0.039	0.030	0.031	<0.006	0.63	≤0.005
Eggs	0.36	0.044	*	<0.005	0.47	≤0.003
Females having hatched eggs	0.18	<0.008	0.029	0.11	0.57	0.036
Newly hatched zoeae	0.11	<0.007	0.021	<0.013	*	0.031
Postlarvae	0.011	<0.007	<0.001	<0.004	<0.005	≤0.015

* Could not be quantitated.

Survival data were treated with a two-way nested analysis of variance (anova) (Sokal and Rohlf, 1969) following arcsine transformation of percent survival. Data for development time were analyzed with two-way nested analysis of variance and covariance including repeated measures (Dixon, 1975) on a DEC system 10 computer. Data for postlarval length were analyzed in the same manner as developmental time.

A Pearson correlation coefficient (Nie et al., 1970) was calculated in order to determine the degree of relationship between postlarval length and developmental time.

III. RESULTS

Survival data for the 36 groups of larvae are presented in table 2. Analysis of variance of the survival data (table 3) indicates that there is no evidence for significant difference in survival of larvae among broods of different females from the same collection site; nor is there significant difference in survival of reared larvae from the different sites ($p > 0.05$).

Data for time required for the larvae to reach metamorphosis are given in table 4. Analysis of variance (table 5) indicates a highly significant variance component ($p << 0.001$) for larval duration among broods reared from different females representing the same site. The variation in larval duration among broods reared from the six different collection sites is not significant ($p > 0.05$).

Length data for postlarvae obtained from the experimental rearings are presented in table 6, and the analysis of variance of these data in table 7. The intrasite (parental) variation again is seen to be highly significant ($p << 0.001$), and the intersite variation is not significant ($p > 0.05$).

Larvae which metamorphosed earliest were generally smaller than those of longer larval duration. Therefore a Pearson correlation coefficient was calculated in order to evaluate the possible relationship between larval duration and length of

Table 2. Survival of reared larvae of *Palaemonetes pugio* from 18 females collected from six sites (F_n = identification number of individual female).

Replicates	Site I Lafayette River			Site II Gloucester Point			Site III Lynnhaven			Site IV Fleeton			Site V James River			Site VI Potomac River		
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}	F_{16}	F_{17}	F_{18}
Bowl A																		
No. of survivors	31	31	30	30	22	28	30	29	23	33	31	31	29	31	30	30	27	20
Percent survival	93.9	93.9	90.9	90.9	66.7	84.8	90.9	87.9	69.7	100.0	93.9	93.9	87.9	93.9	90.9	90.9	81.8	60.6
Arcsine $\sqrt{p}$	75.7	75.7	72.4	72.4	54.8	67.1	72.4	69.6	56.6	90.0	75.7	75.7	69.6	75.7	72.4	72.4	64.8	51.1
Bowl B																		
No. of survivors	30	33	31	32	21	30	27	28	26	30	32	27	31	28	30	28	26	28
Percent survival	90.9	100.0	93.9	97.0	63.9	90.9	81.8	84.8	78.8	90.0	97.0	81.8	93.9	84.8	90.9	84.8	78.8	84.8
Arcsine $\sqrt{p}$	72.4	90.0	75.7	80.0	53.1	72.4	64.8	67.1	62.6	72.4	80.0	64.8	75.7	67.1	72.4	67.1	62.6	67.1

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Table 3. Anova table for survival with arcsine transformation. Estimates of variance components are expressed as percentages (n = 2 bowls; a = 6 sites; b = 3 females).

Source of Variation	df	SS	MS	F_s
Groups (among sites)	5	956.66	191.33	2.36 n.s.
Subgroups (among females)	12	974.34	81.20	2.32 n.s.
Error (within females)	18	631.09	35.06	

$F_{.05}[5,12] = 3.11$

$F_{.05}[12,18] = 2.34$

Variance components:

Error; between measurements on each female $= S^2 = 35.06$ (45.85%)

$$\text{Among females within sites} = S^2_{BCA} = \frac{MS_{subgroups} - MS_{within}}{n}$$

$$= \frac{81.20 - 35.06}{2}$$

$$= 23.07 \text{ (30.16\%)}$$

$$\text{Among sites} = S^2_A = \frac{MS_{groups} - MS_{subgroups}}{nb}$$

$$= \frac{191.33 - 81.20}{6}$$

$$= 18.36 \text{ (24\%)}$$

$$S^2 + S^2_{BCA} + S^2_A = 35.06 + 23.07 + 18.36 = 76.49$$

Table 4. Mean time to metamorphosis in days for larvae of *P. pugio*.

Site No.	Female No.	No. Larvae Metamorphosed	Mean (days)	Standard Deviation
I Lafayette River	1 2 3	61 64 61	13.1 13.5 14.2	0.8 1.2 2.1
II Gloucester Point	4 5 6	62 58 44	12.9 18.1 18.0	1.1 3.0 1.8
III Lynnhaven	7 8 9	57 57 49	17.5 15.4 17.8	1.9 1.3 1.6
IV Fleeton	10 11 12	63 63 58	16.0 15.1 21.7	1.7 1.0 3.2
V James River	13 14 15	60 59 60	15.6 15.6 15.1	1.3 0.9 0.6
VI Potomac River	16 17 18	57 53 48	17.1 14.2 16.8	2.4 1.2 2.1

Table 5. Anova table for time to metamorphosis.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Sites	1,756.84201	5	351.36840	1.3825 n.s.
Females (within sites)	3,049.84500	12	254.15375	80.99806 ***
Error	3,187.98019	1016	3.13778	

n.s. Not significant ($p > 0.05$)

*** Highly significant ($p < 0.001$)

$F_{.05}[5,12] = 3.11$

$F_{.001}[12,\infty] = 2.74$

Table 6. Length (in mm) of postlarvae of *P. pugio* reared from six sites.

Site No.	Female No.	No. of Larvae	Mean Length (mm)	Standard Deviation
I Lafayette River	1 2 3	61 64 61	7.2 7.4 7.3	0.2 0.2 0.4
II Gloucester Point	4 5 6	62 58 43	7.5 7.8 7.6	0.3 0.5 0.6
III Lynhaven	7 8 9	56 57 49	7.8 7.7 7.8	0.4 0.5 0.5
IV Fleeton	10 11 12	63 63 58	7.1 7.0 8.2	0.3 0.2 0.6
V James River	13 14 15	60 59 60	7.4 7.1 7.3	0.4 0.4 0.4
VI Potomac River	16 17 18	57 53 48	7.4 7.0 7.3	0.4 0.3 0.4

Table 7. Anova table for postlarval length.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Sites	41.74599	5	8.34920	1.49366 n.s.
Females (within sites)	67.07723	12	5.58977	34.51303 ***
Error	164.22859	1014	0.16196	

n.s. Not significant ($p > 0.05$)

*** Highly significant ($p < 0.001$)

$F_{.05}[5,12] = 3.11$

$F_{.001}[12,\infty] = 2.74$

postlarvae. The correlation coefficient was 0.51 at the 0.00001 significance level indicating a weak, but significant relationship between time to metamorphosis and size at metamorphosis.

Sample size and extraction and analysis dates for frozen samples analyzed for Kepone are presented in table 8, and the Kepone values for these samples, arranged by size, are given in table 1. These results could not be analyzed statistically because of lack of replication due to small sample size. [Although recovery and reproducibility data could not be obtained for these samples, oyster samples fortified at 0.3 ppm and fish (bream) samples fortified at 0.1 ppm extracted with a Polytron gave recoveries of 84 percent and 80 to 94 percent respectively (M.K. Ward, personal communication).] Nevertheless, table 1 illustrates that samples from sites 1 and 5 (the Lafayette River and James River respectively) exhibited high Kepone levels relative to the other sites. Laboratory reared postlarvae from all sites showed very low (or undetectable) Kepone concentrations.

IV. DISCUSSION

P. pugio is known to be more Kepone-tolerant than several other estuarine organisms. Acute 96-hour toxicity bioassays showed the LC_{50} (expressed in micrograms per liter) to be 6.6 for spot, 70 for sheepshead minnows, 10 for a mysid crustacean, 121 for *P. pugio*, and 210 for the blue crab, *Callinectes sapidus* (Hansen, et al., 1976). At least one other species of *Palaemonetes* has been shown to have populations resistant to a variety of organochlorine, organophosphorous, and carbamate insecticides (Naqvi and Ferguson, 1970).

Chemical analyses of samples revealed Kepone concentrations from the six sites ranging from undetectable to moderately high levels. Intersite ranges were as follows: $\leq$ 0.005 to 0.63 ppm for ovigerous females; $\leq$ 0.003 to 0.47 ppm for eggs; $\leq$ 0.008 to 0.57 ppm for females which had recently hatched larvae; $\leq$ 0.007 to 0.11 ppm for newly hatched zoeae; and $\leq$ 0.001 to $\leq$ 0.015 ppm

Table 8. Extraction and analysis dates, sample weights, and volumes of frozen samples analyzed for Kepone (data courtesy of Virginia Institute of Marine Science).

Site	Sample	Extraction Date	Gas Chrom Analysis Date	Sample Weight (gm)	Sample Volume (ml)
1	Newly hatched zooea	02-08-77	02-11-77	0.65	17.5
	Ovigerous females	09-15-76	09-21-76	3.11	29.5
	Females having hatched eggs	08-04-76	08-04-76	2.70	30.0
	Eggs	07-26-76	07-26-76	1.75	29.5
	Postlarvae	02-08-77	02-11-77	0.20	21.5
2	Newly hatched zooea	02-03-77	02-11-77	1.37	19.5
	Ovigerous females	10-20-76	02-11-77	3.51	29.0
	Females having hatched eggs	10-20-76	02-11-77	2.28	29.0
	Eggs	02-10-77	02-15-77	1.03	21.0
	Postlarvae	02-09-77	02-15-77	0.37	24.0
3	Newly hatched zooea	02-07-77	02-15-77	1.77	21.0
	Ovigerous females	02-01-77	02-15-77	2.88	19.0
	Females having hatched eggs	09-15-76	09-21-76	2.62	29.0
	Eggs	02-10-77	02-15-77	2.07	25.0
	Postlarvae	02-09-77	02-14-77	0.34	22.0
4	Newly hatched zooea	02-03-77	02-16-77	0.85	18.0
	Ovigerous females	02-01-77	02-16-77	2.00	15.0
	Females having hatched eggs	09-08-76	09-09-76	2.89	29.5
	Eggs	02-10-77	02-16-77	0.20	22.0
	Postlarvae	02-07-77	02-17-77	0.24	20.0

(cont'd.)

Table 8. Extraction and analysis dates, sample weights, and volumes of frozen samples analyzed for Kepone (data courtesy of Virginia Institute of Marine Science) - concluded.

Site	Sample	Extraction Date	Gas Chrom Analysis Date	Sample Weight (gm)	Sample Volume (ml)
5	Newly hatched zooea	02-02-77	02-16-77	2.09	12.5
	Ovigerous females	09-06-76	09-09-76	2.08	30.0
	Females having hatched eggs	09-06-76	09-09-76	1.90	29.5
	Eggs	09-06-76	09-09-76	1.85	29.5
	Postlarvae	02-09-77	02-17-77	0.22	22.0
6	Newly hatched zooea	02-02-77	02-16-77	1.08	20.0
	Ovigerous females	09-07-76	09-09-76	2.85	29.5
	Females having hatched eggs	09-07-76	09-09-76	3.82	29.5
	Eggs	02-10-77	02-16-77	1.24	25.5
	Postlarvae	02-09-77	02-17-77	0.05	17.0

for laboratory-reared postlarvae. Not surprisingly, the highest levels were found in samples from the heavily contaminated James River and the second highest levels from the Lafayette River, located near the mouth of the James. While detectable amounts of Kepone were found in at least some samples from the other sites, the levels were generally low, and the populations remote from the James may be considered relatively uncontaminated. In spite of these wide ranges in concentrations we were able to detect no difference in larval response (lethal or sublethal) attributable to the geographic origin of the larvae. However, due to the limitations of the Kepone analytical technique (minimum sample size = 1 gram), individual females from which larvae were reared could not be analyzed for Kepone concentration. The analyzed samples were a composite of several animals collected from the same population, and the determined concentrations were average values. There is, therefore, no way to trace each laboratory-reared brood to a specific parental Kepone concentration. Thus it is possible that the reared larvae from a given collection site may have arisen from females in which the Kepone levels were not representative of the general population.

As mentioned earlier, this study was occasioned by the occurrence of erratic results in larval rearings from an area known to be contaminated. The erratic results were not obtained, and therefore were not explained, by this study. The purpose of our field sampling was simply to determine whether the areas from which the reared animals were collected were subject to Kepone contamination. A statistical sampling plan was not attempted, but clearly should be in order to ascertain representative contamination levels and individual variability in contamination of animals obtained from a given area. We cannot eliminate the possibility that larvae reared for this study came from relatively "clean" individuals, and hence exhibited no immediately observable effects.

The temperature and salinity combination used for the larval rearings in this study has been determined near optimum for this species, at least for stocks from lower Chesapeake Bay (Floyd, personal communication), and the larvae always received sufficient uncontaminated food. Hence the laboratory-reared larvae were relatively unstressed. Field hatched larvae from eggs, or females with Kepone residues near the highest reported here, might be subjected to environmental stresses, such as temperature and/or salinity extremes, contaminated food, etc. Thus synergistic effects, unexamined here, may be operating under field conditions. In addition, although the Kepone concentrations detected in this study may not have had direct observable effects on larval development of *P. pugio* reared in the laboratory, there may well be biomagnification effects upon predators of this species, a topic outside the scope of this study.

Although this experiment was not designed specifically to study parental variation, highly significant differences in larval development time and postlarval length were found among broods from single sites. If we had not accounted for variation due to parentage (by rearing broods separately) we might have falsely concluded that that variation was due to site of origin.

V. CONCLUSIONS

Larvae of *Palaemonetes pugio* obtained from three adults collected at each of six sites within Chesapeake Bay, when reared under controlled laboratory conditions, showed no significant differences in larval survival, larval duration, or length of postlarvae attributable to site of origin.

Egg-bearing adults of *P. pugio*, females which had recently hatched larvae, eggs, newly hatched larvae, and laboratory-reared postlarvae, when analyzed for Kepone, showed variation in concentration ranging from undetectable to levels of 0.6 ppm. Populations from the James River and nearby Lafayette River showed the highest concentrations of Kepone; distant populations showed lower levels. Laboratory-reared postlarvae representing all six populations had very low or nondetectable Kepone concentrations.

Survival rates among broods from individual females ranged from 61 to 100 percent, but did not differ significantly among females from any one site; nor did the mean survival among larvae differ from site to site.

Mean time to metamorphosis ranged among broods from 12.9 to 21.7 days. Parental variation in this parameter was highly significant, whereas site variation was not.

Mean postlarval length which ranged from 7.0 to 8.2 mm exhibited significant intrasite variation, but intersite variation was insignificant.

There was a weak, but significant relationship between time to metamorphosis and postlarval length at metamorphosis.

VI. RECOMMENDATIONS

1. For successful continuation of analysis of Kepone in *P. pugio*, the previously described Polytron extraction method should be evaluated for this substrate, including recovery and reproducibility studies. Larger sample size would probably enhance extraction efficiency. Other methods, e.g., extraction utilizing a micro-Soxhlet assembly, should be examined. Finally, additional modified means of sample cleanup may be necessary for future routine analysis (M.K. Ward, personal communication).

2. Acute toxicity tests and long-term toxicity tests should be determined on larvae in order to determine at which concentrations Kepone has adverse effects on young stages of *P. pugio*.

3. The present study showed significant variation in responses of larvae from different females. Obviously such effects should not be ignored in bioassay design. A study of effects of parentage on variation in larval responses for *P. pugio* should be conducted in order to establish the minimum number of females which would allow the most reliable representation of populational response.

4. A statistically designed sampling plan should be carried out in order to determine representative contamination levels and individual variability in contamination of animals living in a given area.

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Start→ Fate and Effects of Kepone^R in Artificial Estuarine Ecosystems. A. W. BOURQUIN,* P. H. PRITCHARD and H. L. FREDRICKSON. U.S. Environmental Protection Agency, ERL, Gulf Breeze, Fl. 32561.

Fate and effects of the pesticide Kepone were studied in artificial ecosystems, containing water and sediment from either Range Point salt marsh, FL or the James River, VA. Approximately 75-80% of ^{14}C Kepone added (0.5 ppm) to the systems accumulated in the detrital fraction. Using high pressure liquid chromatography and GC-mass spectral analysis, we detected no transformation products with a variety of experimental regimes including anaerobic or aerobic conditions. Neither the addition of glucose (0.1%) nor naphthalene (0.1%) stimulated the transformation of the pesticide in analogous systems. No $^{14}CO_2$ was produced in any experiment. James River sediment with a history of Kepone exposure was likewise ineffective.

Effects of Kepone on microbial communities in these artificial ecosystems were monitored by determining the rate of CO_2 evolution and metabolite accumulation from ^{14}C-methyl parathion (MPS). James River sediment systems were more active than Range Point sediment system in the metabolism of MPS to CO_2. The presence of Kepone at concentrations of 0.6 mg/kg of sediment in either sediment system reduced the degradation rate of MPS by 59% and 54%, respectively. These studies indicate that Kepone, although resistant to microbial attack, can be inhibitory to the microbial community responsible for the degradation of other pollutants in an artificial ecosystem.

American Chemical Society National Meeting
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THE FATE OF 14C-KEPONE IN ESTUARINE MICROCOSMS. R.L. Garnas, A.W. Bourquin, and P.H. Pritchard. U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561.

Following the contamination of the James River with Kepone, laboratory data concerning its fate in the estuary were necessary for corrective actions and mathematical modeling efforts. The movement and transformation potentials of 14C-Kepone were studied in static and continuous flow estuarine microcosms. Biotic and abiotic transformation and volatilization of the chemical were not apparent in these studies. Following its adsorption from water in these model systems, Kepone desorbed from salt marsh sediments and James River sediments. While this desorption was independent of environmental water temperatures and salinities in sediment-water systems, the Kepone concentration in the water column was proportional to its concentration in sediment. Some James River sediments retained high levels of radiolabeled chemical following conventional solvent extraction.

Burrowing polychaetes (*Arenicola cristata*) were added to salt marsh sediment in larger continuous flow systems to define the effect of macrobenthic biota on the fate of Kepone. These polychaetes accumulated high residues of Kepone and died; although the tissues were allowed to decompose in the system, the accumulated Kepone was not as available for desorption and washout from the system as compared to Kepone adsorbed to sediment.